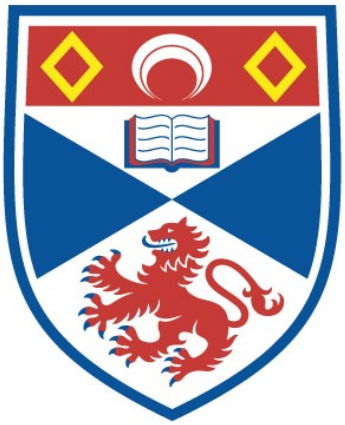
POLLINATION ECOLOGY OF CULTIVATED AND WILD RASPBERRY (RUBUS IDAEUS) AND THE BEHAVIOUR OF VISITING INSECTS

Ali A. M. Bataw

A Thesis Submitted for the Degree of PhD
at the
University of St Andrews



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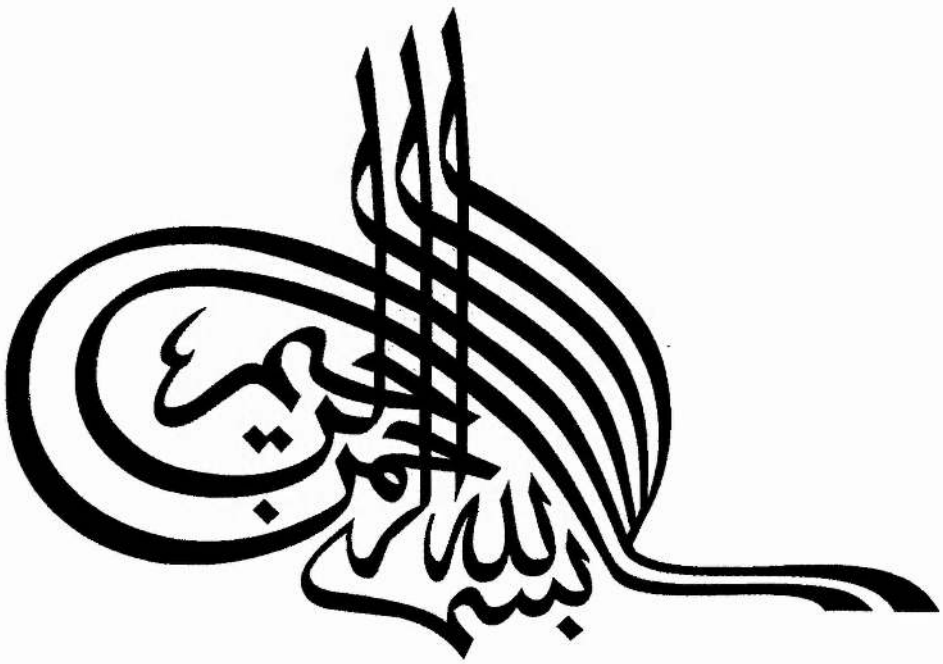

In the name of Allah, the Beneficent, the Merciful

واوحى ربك الى النحل ان اتخذي من الجبال بيوتا ومن الشجر
ومما يعرشون . ثم كلي من كل الثمرت فاسلكي سبل ربك ذللا يخرج
من بطونها شراب مختلف الوانه فيه شفاء للناس ان في ذلك لاية لقوم
يتفكرون . (النحل ٦٩-٦٨)

صدق الله العظيم

And thy Lord taught the Bee to build its cells in hills, on trees, and in [men's] habitations; Then to eat of all the produce [of the earth], and find with skill the spacious paths of its Lord; there issues from within their bodies a drink of varying colours, wherein is healing for men: Verily in this is a sign for those who give thought. [The Holy Qur'an, Ch 16: verses 68-69]

Declaration

I, Ali A. M. Bataw, hereby certify that this thesis has been composed by myself, and that it is a record of my own work, and that it has not been accepted in partial or complete fulfillment of any other degree or professional qualification.

I was admitted to the Faculty of Science of the University of St. Andrews under Ordinance General No. 12, and as a candidate for the degree of Ph.D., on 1 October 1991.

I hereby certify that the candidate has fulfilled the conditions of the Resolution and Regulations appropriate to the degree of Ph.D.

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I would like to take this chance to extend my thanks to the members of both my own and my wife's large families back in Libya; and especially our fathers and mothers for their encouragement, support and hardship during our long stay in the UK. Finally, this work would never have been done without the help, patience and care of my wife Fathia, my daughter Hajer and my son Abdulgader throughout the entire period.

Pollination ecology of cultivated and wild raspberry (*Rubus idaeus*) and the behaviour of visiting insects

Abstract

Floral morphology and anthesis were studied in commercial and in wild populations of raspberry (*Rubus idaeus*). Young flowers offered both nectar and pollen, but medium and old flowers offered nectar only, in diminishing quantity. All the three types had similar ageing patterns and diurnal patterns of nectar secretion, but they differed significantly in the nectar standing crop. Variation in nectar secretion rates within the raspberry cultivars was examined; Glen Moy produced more nectar per flower, and more flowers per meter, than Glen Prosen and wild raspberry flowers. There was significant daily variation in secretion rate, individual flowers in all cultivars showing variable rates of secretion even on the same branch. Time of sampling, effects of insect visitors, flower age and weather conditions all showed significant relationships to nectar availability.

The three raspberry types have in common certain insect species as visitors, the most abundant being bumble bees (*Bombus lapidarius*, *B. lucorum*, *B. terrestris*, *B. pratorum* and *B. pascuorum*), *Apis mellifera*, *Andrena* species and hover flies. Bumble bees were responsible for about 60% of all visits, with honey bees, *Andrena* and hover flies making up most the remaining visits. *Bombus* species were more abundant through the particular observation days and through the different seasons, and they were present at almost all times of observations irrespective of climatic conditions in the studied area.

The foraging behaviour and activity patterns, pollen loads and pollinating efficiency of the *Bombus* spp., *Apis* and *Andrena* spp. were analysed in relation to plant phenology, anthesis and dehiscence and to climatic variables. All bees had substantial pollen deposited on their bodies during visits, though few specifically

collected it. *Bombus* species were found to strongly select young flowers, especially early in the morning when pollen was most abundant, while *Apis* and *Andrena* species visited unselectively. Bumble bees also foraged over substantially longer periods of the day, and in poorer weather, and they visited more flowers per minute than *Apis* and *Andrena* species. *Bombus* species also carried more pollen grains on their bodies than *Apis* and *Andrena* species, and deposited more pollen on raspberry stigmas; and because they foraged over longer range, they transferred pollen grains for longer distance than *Apis mellifera.*

The flight directionality of *Bombus*, *Apis* and *Andrena* species among the flowers of Glen Moy and Glen Prosen was analysed. Pollen flow was also studied using fluorescent dyes, in field experiments during 1993 and 1994. *Bombus* and *Apis* transferred dye particles (pollen mimics) to different extents in different directions in the field. All the three visitors showed a strong tendency to move in the south-north direction (the direction of the raspberry rows); this would lead to increase in the gene flow within the same row in the presence of pollen carry-over. Pollen was carried up to 60m by *Bombus* species and 35m by honey bees.

The work presented in this thesis provides evidence that (at least in Scotland) bumble bees are likely to be more important as pollinators of raspberries than other visitors. Reason why *Bombus* may be the preferred insect pollinators in wild and cultivated areas are discussed.

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1.1. Background information about raspberry

Rubus is one of the most diverse genera in the plant kingdom. It contains a wide spectrum of wild species in addition to those from which domesticated cultivars of edible fruits have been selected (Darrow 1937). Focke (1910-1914 cited in Jennings 1988) divided the genus into 12 subgenera: raspberries belong to the subgenus *Idaeobatus*, whose species are distinguished by the ability of their mature fruits to separate from the receptacle. Red raspberries are widely distributed in all temperate regions of Europe, Asia and North America. Over 200 species are recognised; several of them have been domesticated, of which the most important is the European red raspberry (*Rubus idaeus* L.) (Darrow 1937; Jennings 1988). Red raspberry was introduced for the first time into cultivation in Europe about 400 years ago. Three hundred years ago there were at least two varieties cultivated in England (Darrow 1937).

The wild raspberry is distributed throughout Great Britain, except for the fen areas. In Ireland its frequency decreases from the north-east to the south-west. Individual canes in wild forms are shorter, hairier and thinner than in cultivated forms, and they have shorter and thinner laterals bearing small flowers. The fruit size is easily distinguished between cultivated and wild plants, the fruit size of cultivated raspberry being two to three times as large as that of the wild form (Haskell 1960).

The flowers of commercial raspberry cultivars are mainly hermaphroditic (Jennings 1988). Most red raspberry cultivars grown in Europe are fully self-fertile and thus self-compatible (Keep 1968). In the early days of raspberry domestication, the improvement in fruit size or yield was the main target. Later the need changed, with selection for vigorous cultivars able to face viral infections; there was also selection to satisfy particular demands, such as suitability for processing and travel purposes (Jennings 1988).

Many varieties were produced and cultivated in Britain as well as the rest of the world. In Scotland many varieties were bred at the Scottish Horticulture Research Institute and the main release was Glen Clova, now grown widely in eastern Scotland (Jennings 1988).

Glen Moy and Glen Prosen were the first spine-free raspberries produced in 1981 by the Scottish Crop Research Institute. Glen Moy is an early cultivar and Glen Prosen is a late cultivar (Jennings 1988).

The raspberry flowers generally have five petals with numerous stamens and styles, typically about 90 of each (Redalen 1980; Jennings 1988). Each style is attached to an ovary, which develops as a fleshy drupelet after fertilisation. Outside the rings of reproductive organs is a further substantial ring of nectary tissue, the product of which is freely exposed to visitors, since the petals become fully reflexed whilst the styles and stamens stay erect (Jenning 1988). The stamens diverge so that even short tongued insects can reach the nectar, and visiting bees will touch both stamens and stigmas in nearly every flower visited (Free 1993). Pollen grains vary in size and pore number (Jennings 1988; Haragsimova-Neprasova 1960).

1.2. The importance of insect pollination in red raspberry.

Although red raspberry is known to be self-fertile, much evidence exists to suggest that the presence of pollinating insects will increase the size and number of berries, and also produce a more symmetrical shape (Johnston 1929; Couston 1963; Shanks 1969; Free 1993). Thus insect pollination can increase the yield and also the quality of a raspberry crop (Benedek 1983; Winston & Graf 1982). When insect pollinators are present fruit are less likely to be deformed (de Oliveria *et al* 1983).

Colbert & de Oliviera (1990) showed that cross-pollination produces substantially heavier fruits than selfing, and that raspberry also exhibits clear metaxenia (the phenotypic manifestation of a development arising from maternal tissues (improved fruit quality) after fertilization by pollen from other varieties). Wieniarska (1987) investigated the yield of four red raspberry cultivars in conditions of open pollination and without pollinating insects, and found that the yields of open pollinated plant were always significantly higher than those without insects.

Eaton *et al* (1968) suggested that each flower of red raspberry needs to be visited for at least a four day period, for a maximum drupelet set and a well-formed berry. The drupelet number and berry weight increase proportionally with the number of bee visits, and five or six visits per flower are reported to be sufficient to attain adequate pollination and fruit development (Chagnon *et al* 1991).

1.3. Flowering time and pollination

Flowering includes floral bud initiation and development, blooming (anthesis), and floral persistence. Flowering time is a trait which could be critical to a plant's success through its effect on reproductive processes such as pollination and the timing of seed dispersal. Optimal flowering time may be a trade-off between a variety of selective factors, one of which is pollinator availability (Waser 1978). The selective forces that affect flowering phenology have been debated extensively (eg. Bawa 1983). Particularly controversial has been the idea that the flowering phenology of individual species is linked to the abundance of pollinators, and competition for those pollinators with other plant species, an idea dating right back to Robertson (1890). Some evidence does indicate that the seasonal availability of pollinators may select for certain flowering times in animal-pollinated species, and many studies show seasonal correlations between pollinators and flowering time (e.g. Mosquin 1971; Bawa

1983). Abiotic factors are often correlated with flowering time and may limit flowering seasons either directly by affecting the ability to produce flowers or indirectly by affecting pollen vectors. Biotic factors, such as parasitism, can influence flowering time, but these effects have received little attention (Rathcke 1985).

Willmer *et al* (1994) during an investigation of two varieties of raspberry in East Scotland indicated that the seasonal flowering was very dependent on environmental variables.

1.4. Nectar production

Nectar production plays a vital role in the pollination of most flowering plants. It has therefore been assumed that the sugar constituents are a major factors in determining the attractiveness of flowers to pollinators (Faegri & van der Pijl 1979; Southwick *et al* 1983). Much work has been done concerning the energetics of plant-pollinator interactions. Often these studies deal with measurement of volume of nectar, sugar concentration and total energetic value of nectar produced by individual flowers (Bolten *et al* 1979). Pollination is successful in many plant species as a consequence of pollinators seeking out nectar (Southwick *et al* 1981). Yet few data are available for raspberry regarding the factors which influence the volume and concentration of nectar present in the flowers at any time; the secretion activity of nectaries; the equilibration with the weather; and the effects of repeated removal of nectar by insects in different ages of raspberry flowers.

Removal of nectar from flowers affects the process of secretion, probably due to the differences in the osmotic relations of the nectar tissues and the secreted nectar (Raw 1953). Because the nectaries of raspberry are quite exposed, the quality of nectar they contain is potentially greatly influenced by

changes in relative humidity (Free 1993; Corbet *et al* 1979a); in particular, nectar concentration responds to fluctuations in relative humidity of the atmosphere (Corbet 1978a, Corbet *et al* 1979b; Southwick *et al* 1981). In some plants nectar volume and nectar concentration increase directly with the age of the inflorescence (Wood 1961) but in other plants the nectar production decreases with increase in age (Southwick & Southwick 1983).The nectar may also change in amount and in composition through the day and from one day to another (Corbet 1978b).

During anthesis in *Rubus idaeus* all nectar secretion can be related to the entomogamous nature of pollination (Said & Nesme 1982). *Rubus idaeus* is an abundant species, which offers almost unlimited supplies of nectar during at least part of its flowering season. The average amount of nectar exuded per flower in the red raspberry varieties studied by Whitney (1984), during 24 hr periods, was found to vary between 3.8 and 14.1 µl, the sugar concentration from 37.8 to 59.2%, and the sugar amount from 1.9 to 6.7 mg. As the flower age increased, the nectar exudation decreased, while the percentage sugar content changed only slightly. In another study, daily nectar exudation proved incessant and its intensity was enhanced in the morning and afternoon hours (Simidchiev 1976).

However, productivity varies between cultivars. For example, Petkov (1963) investigated two varieties of raspberry in the area of the town of Sofia, where he found the amount of nectar produced by the blossom of the Marlboro variety was 15.9 mg per day and the nectar concentration varied from 30 to 60%. The nectar produced by the Newburgh variety was 20.4 mg per day, with nectar concentration varying from 32 to 64% (temperature 15.5 - 16.2 °C).

1.5. The role of environmental factors in nectar secretion

There is abundant evidence for effects of the environment, or of the physiological status of the plant, on nectar both before and after secretion. However, the general lack of detail makes it difficult to evaluate. Synergistic effects of factors such as nutrient level, water status, temperature, radiation and plant age, contribute much to the large variance in the measurements of nectar secretion. In many cases, the quantities of nectar secreted by the nectaries have been assumed to depend only on the interaction with pollinators, disregarding other sources of variation. This approach, although it takes into account the evolutionary history of the relationship, often ignores the variation in nectar characteristics attributable to physiological or environmental factors (Corbet *et al* 1979b).

Nectar flow is dependent upon the plant, the environment, and the interactions between the two (Pedersen 1961). External factors influencing secretion are those of weather and soil, each of which is a complex of interrelated factors, and it is often difficult to separate out the individual components in field observations (Shuel 1975).

Temperature has received more attention than any other factor and there is a difference of opinion regarding its importance. Records of daytime temperatures may reflect conditions of sunlight which themselves can cause wide variation in nectar flow (Shuel 1967). The effects of temperature on nectar secretion were noted long ago by Bonnier (1879), Wilson (1881), and Kenoyer (1917). Many researchers have shown that temperature seems to act as a critical threshold agent (Vansell 1940; Pederson 1953). From a study with nine different species, Huber (1956) concluded that temperature was the factor most closely related to nectar secretion, and the same conclusion was reached by Zauralov (1979). Temperature is known to affect rates of photosynthesis and translocation (Canny 1973; Marowitch *et al* 1986). Southwick (1984) showed that during the

flowering period, a high proportion of the daily photosynthate assimilated by plants goes into nectar.

Several authors (Shuel 1975; Corbet *et al* 1979a,b; Southwick *et al* 1981) have found that humidity has a pronounced inverse effect on nectar sugar concentration. It is likely that this effect is chiefly physical, operating in the following manner. As nectar is secreted, it begins to undergo a change in concentration until its vapour pressure comes to equilibrium with that of the atmosphere. Unless the humidity of the atmosphere is very high, the change will be a loss of water molecules to the air and an increase in sugar concentration. Rates of increase in nectar sugar concentration can be extremely rapid in flowers in which the nectar is exposed (Percival 1946; Fahn 1949; Bertsch 1983; Free 1993).

However a direct effect of atmospheric humidity on secretion has not been established. Evaporation is hastened by high temperature and rapid air movement across the nectaries. Also evaporation is more rapid from a thin film of nectar than from large globules (Shuel 1955a,b; Corbet *et al* 1979b). Individual investigations involving climatic conditions for several crops has been extensively reviewed by Beutler (1953), Percival (1965), and Corbet *et al* (1979a,b).

In general, conditions which impose no appreciable limitations on growth and which promote a reasonable balance between vegetative and reproductive development seem to support good nectar production (Shuel 1967).

1.6. Pollen production

Pollen is often the only resource collected by bees from some flowers. It is gathered extensively from flowers that produce no other resource. Pollen

release is variable in its initiation, peak occurrence, and duration. This is due to genetic control and also the influence of weather (Stanley & Linskens 1974).

Stanley & Linsken (1974) point out that anther dehiscence frequently occurs over a longer time period than the receptive period of stigmas on the same plant. Most plants dehisce in the early morning or at two peaks during the day, but dehiscence is nocturnal in others. Anther sacs normally release pollen due to the breakdown of endothecial cells, caused by the action of intercellular enzymes, ambient temperature, and humidity (Stanley & Linsken 1974).

Many investigators have reported that temperature is the most important climatological factor influencing dehiscence of the pollen sacs in some plants, and dehiscence may occur at any time of day when the proper temperature is reached (Seaton & Kremer 1938). Humidity apparently was not operative in influencing the time of anthesis or dehiscence in cucumber flowers; and even though the time of day was a controlling factor for both anthesis and anther dehiscence, it undoubtedly was modified by temperature, since a highly significant positive correlation was found between temperature and anther dehiscence (Seaton & Kremer 1938).

Dehiscence occurs gradually over several hours or even days in species having pores through which pollen is ejected (Buchmann 1983). In other plants, the most common type of dehiscence involves mass presentation of pollen on the surface of the anthers through slitlike openings (Proctor & Yeo 1973; Stanley & Linskens 1974; Faegri & van der Pijl 1979; Crepet 1983).

1.7. Visitor abundance and diversity.

There appears to be a close relationship between quantity and concentration of sugar in nectar, and bee visits. Butler (1945) stated that in a population of bees the greatest number will work the plant species in which the

nectar is most abundant and most easily obtainable, provided concentrations are about the same in all species. Vansell (1934) and Wykes (1952) found that sugar concentration was an important factor in determining which species of plant bees would be working at most freely for nectar.

Butler (1945) mentions that the nectar concentration largely determined the plant species which honey bees visited, but he felt that the population determinant was abundance of nectar.

Rubus idaeus is attractive to a wide range of pollinators (Whitney 1984). Raspberry flowers are accessible to many types of insect visitors (Faegri & van der Pijl 1979), and this factor, coupled with the high level of nectar and pollen production and wild distribution of *Rubus idaeus*, should ensure that a varied insect fauna visit the bloom (Hansen & Osgood 1983).

Winston and Graf (1982) found low abundance and diversity of native bee pollinators on berry crops in the Fraser Valley in British Columbia. Almost all bees collected were bumble bees (sub-family Bombinae); only four species of solitary bees (comprising 5% of the total) were found. In Washington State large numbers of honey bees (*Apis mellifera*) were always observed in raspberry fields (Shanks 1969). Although a few wild bees of various species were found on raspberries in Washington State, the honey bee was by far the most common bee on them, and probably their most important pollinator (Primack & Silander, 1975). de Oliveira *et al* (1983) stated that *Apis mellifera* was responsible for 76% and 97% of the flower visits to red raspberry in 1978 and 1979 respectively in the Quebec area. Other important pollinators in that study were *Andrena* species (Andrenidae), Bombinae, Halictidae and Syrphidae. In East Maine, Apoidea were considered probably the most responsible for the pollination of the *Rubus idaeus* because of their behavioural and morphological adaptations for pollen transport (Hansen & Osgood 1983). Ferrazzi & Botasso (1989)

mention that *Apis mellifera* foraged mainly on wild and cultivated raspberry in Italy, and appeared to be very useful for raspberry pollination. Thus most studies to date have emphasised the importance of *Apis*, with *Bombus* only taking precedence in the Canadian site

1.8. Foraging behaviour

The foraging behaviour of pollinators is a function of resource quality, (which is expressed in terms of plant density and quality of reward per plant (Levin 1978)), and may in turn be affected by the quantity and distribution of the nectar sugar rewards offered by the flowers (Heinrich & Raven 1972; Heinrich 1975a). Pedersen (1961) stated that the nectar-collecting honey bees visiting alfalfa are influenced by the quality and quantity of the nectar as well as perhaps by other factors. Ciurdarescu (1971) showed that the variation in number of alfalfa pollinators was directly proportional to the air temperature and inversely proportional to the relative air moisture. All insects, including pollinators, are dependent upon microclimatic conditions (Willmer 1982). Bees are unusual in having endothermic capabilities, but still exhibit specific minimum temperatures for activity (Stone & Willmer 1989) This means that each species of bee or fly has ''microclimatic window'' (Corbet *et al* 1993), in which it can effectively behave as a pollinator. Foraging behaviour is affected directly by weather conditions. Bee flight activity is strongly affected by abiotic factors such as temperature, rainfall and wind (Thorp 1979). The influence of weather conditions on foraging activity of *Apis cerana indica* was specifically investigated by Sihage (1984). He indicated that fluctuations observed in pollen gathering activity were correlated with the variations in relative humidity and amount of rainfall. Humidity and rainfall showed a positive significant relationship with pollen gathering activity but had no effect on nectar gathering activity; and foraging activity was not significantly affected by temperature or wind speed (Sihag 1984).

Honey bees were more numerous than bumble bees on raspberry, and their numbers fluctuated more, in a study by Free (1968). Bees collecting nectar from raspberry flowers acquired pollen incidentally; more than half of the bees packed it into the pollen basket, and the others discarded it. Honey bees spent 9 sec/flower when collecting pollen and 8 sec when collecting nectar only, while bumble bees worked slightly faster (Free 1968, 1993). Again this varies between cultivars; for example Petkov (1963) observed that honey bees spent 11 sec/flower on one raspberry variety, and 13 sec/flower on another.

Some bees collect either pollen or nectar on a single trip. However, other bees may collect both materials on the same trip. The reasons why certain bees collect both at the same time are not known (Shaw *et al* 1954).

For effective pollination the population of honey bees in a raspberry field should be high enough and well distributed in order to lead to an increase in the number of drupelets (Eaton *et al* 1968). The stigmas and anthers of raspberry flower mature over several days, and repeated pollinator visits are therefore required to achieve maximum fruit set. One bee visitor for each 100 open raspberry blossoms appears desirable, but again this figure depends on other factors such as weather conditions (McGregor 1976).

1.9. Pollination efficiency

The pollination efficiency of visitors will depend on many factors including their constancy, and their effectiveness in transferring conspecific pollen to the stigma (Bohart and Nye 1960). Efficiency can be measured: by quantity and placement of pollen grains on the visitor's body using laboratory analysis; or by filming the foraging behaviour of the visitors (Macior 1967). Further useful data include visitor's activity patterns, their abundance, and the

pollen load deposited on receptive stigmas (Bohart & Nye 1960; Levin & Berube 1972; Primack & Silander 1975; Yeboah Gyan & Woodell 1987).

The exact location of pollen on a pollinator's body may indicate whether the pollen will reach its target or be wasted (Dafni 1992). The floral morphology of *Rubus idaeus* ensures that insect visitors with any pubescent ventral surfaces or head regions can also bring about significant pollen transfer (Faegiri & van der Pijl 1979).

The constancy of a bee to one plant species can be judged either by observing it directly or by analysing its pollen loads. Constancy has mostly been judged by examining pollen load, although this method gives no information about the behaviour of bees collecting nectar only. It has been concluded that, during a single foraging trip, bumble bees are not very constant and certainly less so than honey bees, and it is to be expected that their day to day constancy would also be less (Free 1993; Yeboah Gyan & Woodell 1987).

1.10. Gene flow

Gene flow, the movement or dispersal of genes within or among populations, is a crucial factor in determining the structure and cohesiveness of species and populations. In plants gene flow can occur via seed or pollen dispersal. Actual gene flow via pollen is found to be greater than would be inferred from pollinator movement alone (Schaal 1980). Plant population geneticists vary in their assessments of the importance of gene flow for plant evolution; the common view is that gene flow is highly restricted (Ehrlich & Raven 1969; Levin 1981). At the other extreme forest geneticists frequently consider gene flow to be extensive (Muona 1990). A third, relatively new, view is that gene flow in plants is idiosyncratic, ranging from very low to very high and varying among species, populations, individual plants and even over a season (Hamrick 1987).

Pollen dispersal and pollen mediated gene dispersal in flowering plants are effected primarily by animals and air currents (Levin & Kerster 1969). Because of the difficulties in measuring the distance to which pollen or seed is dispersed, clear information on gene flow in plant populations is quite limited (Levin & Kerster 1974). It is difficult to measure the pollen flow directly, owing to the lack of distinguishable pollen phenotypes; however, a number of methodologies have been used. Radioactive reagents, coloured dyes or micronized fluorescent dust applied to the anther have been used to estimate pollen flow distance (Simpson, 1954; Turpin & Schlising 1971; Linhart, 1973).

Four general approaches are used to estimate gene flow by pollen: 1) measuring pollen dispersal from a point source; 2) measuring gene dispersal from point and block sources; 3) inferring gene flow from natural population genetic structure; 4) analysis of progeny in the sink population. Gene flow has most frequently been estimated by the first two methods (Ellstrand 1992), by assessing pollen flow. This movement can be measured either indirectly from pollinator foraging distance (Schmitt 1980) and the dispersal of pollen analogues (Campbell & Waser 1989) or directly with marked pollen (Thomson *et al* 1986). Pollen marking techniques provide information on travel distance, knowledge essential for the understanding of the genetic structure of the population (Dafni 1992). In gene dispersal from point and block sources, experimental populations are created by using source plants bearing a genetic marker surrounded by normal plants (Handel 1983).

Measuring dispersal around a source almost always truncates the actual dispersal curve, excluding long distance dispersal events and giving an illusion that dispersal stops at the edge of the study site (Ellstrand 1992). Measuring dispersal from a given plant at two or three different times, it is possible to estimate the distance to which pollen is carried and the number of plants which

receive pollen for the whole population. Pollen may travel long distances, a phenomenon which is well documented. But little information is available on intraspecific pollen travel between individuals (Dafni 1992).

Two aspects of pollinator foraging behaviour have particular importance for patterns of plant gene dispersal. First, pollinator flight distance between plants will determine the distance over which pollen is transferred; second, in self-compatible plants the number of flowers visited per plant will determine the proportion of seeds set that are selfed or out-crossed, and thus will affect the level of inbreeding (Schmitt, 1980) .

The simplest way to estimate pollen transfer distance for animal pollinated species is to record distance flown by pollinators (Price & Waser, 1979; Schmitt 1980; Waddington 1981; Waser & Price 1982). Flight distance between plants is the component of pollinator foraging behaviour most directly affecting plant gene dispersal (Pyke 1978a). An early study on pollinator flight patterns in *Liatris aspera* showed that plant density and spacing control the feeding-flight behaviour of bees and the movement of pollen which they bear (Levin & Kerster 1969).

However, the pattern of pollinator movement is also a fundamental component of pollen dispersal among plants (Levin & Kerster 1974). Relatively quick and erratic flight patterns could be expected to enhance efficiency of pollen movement in a crop (Danka *et al* 1990). But a high frequency of flights within a single plant between adjacent flowers will tend to reduce out-crossing gene flow (Danka *et al* 1990; Kangasjarvai & Oksanen 1989).

1.11. The objectives of the present study

Numerous studies of the science and technology of raising red raspberry have been published; but pollination, a key factor in seed production, has received little attention (Couston 1963; Free 1993). Furthermore, even though wild *Rubus idaeus* L. is described in British Floras, populations of the raspberry wild in Britain have not been studied (Haskell 1960).

From all the articles cited above it is clear that investigations have concentrated on the role of *Apis mellifera* as the main pollinator of red raspberry, with some investigation of other bees. However the role of other insects, particularly bumblebees, some solitary bees and hoverflies has been largely ignored. The aims of the present thesis are:

a) To survey the abundance, diversity, seasonality, and bionomics of insect visitors, and to determine their role as pollinators of wild and cultivated raspberries. To assess the effectiveness of different groups of insect visitors as pollinators of cultivated and wild raspberry, and to investigate whether the cultivars share common insect pollinators and whether the insect pollinators differ in their response to the different raspberry cultivars.

b) Also answers to the following questions are sought. Do raspberry varieties differ in flowering phenology? Do diversity and abundance of pollinators change during the day and season? Do varieties of raspberries differ in nectar production, sugar concentration of nectar, and attraction for insect visitors? Does removing nectar from flowers have an effect on nectar secretion? What are the effects of varying microclimate on the pattern of nectar secretion within days ?

c) Gene flow, by pollen moving among plant populations, can be an important force that maintains the genetic integration of a species. In this study I will measure pollen movement by insect pollinators between cultivated

raspberry crops, with a view to assessing the impact of the possible release of genetically manipulated crops. Staff at SCRI are developing manipulated (thornless) material, and the possible spread of this trait into wild populations must be assessed before the genetically altered material can be released.

Chapter 2
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2.1. Research sites

In this study I used data on raspberry plants and insect pollinators collected primarily from two sites during 1992, 1993 and 1994: dates and times (British Summer Times) are given in the text. The two sites are located in Eastern Scotland. Observations and experiments dealing with cultivated raspberry were carried out at the Scottish Crop Research Institute (S.C.R.I.), Invergowrie, Tayside, Scotland. All field work dealing with wild raspberry was carried out in a diverse wild plant area at Cameron Reservoir.

2.1.1. Scottish Crop Reseach Institute

The cultivated raspberries were planted in 1984 and not sprayed with any insecticides since that date. The field was on a slightly sloping site, south-facing, with rows of canes running North-South. It contained four blocks of raspberry (Fig 2.1), each sub-divided randomly into 24 smaller blocks, 6 each of four cultivars. Glen Moy, Glen Prosen, Glen Clova and Malling Orion, all being hermaphrodite varieties (Jennings 1988). Observations and records concern only the first two of these.

2.1.2. Cameron Reservoir

All field work dealing with wild raspberry was carried out at Cameron Reservoir, about 3 miles west of St. Andrews. The site was covered with diverse plants and surrounded by forest trees. Many bushes of raspberry were scattered along the site Three big bushes of wild raspberry were chosen (7 x 14 , 3 x 7 and 3 x 2 m) on a straight line about 10 meters away from the west shore of the reservoir.

2.2. Floral structure

In this thesis I use the word ''variety'' or ''cultivar'' loosely, for convenience, to refer to all three raspberry types studied.

2.2.1. Flowering phenology

Flowering includes floral bud initiation and development, blooming (anthesis), and floral persistence. Here I generally limit my study to the blooming period, which is the time for pollination. I use the term flowering for this period only in this thesis, unless I indicate otherwise. To follow the flowering sequence for each cultivar, I selected 1m lengths of canes marked them off at the beginning of each season, where growth was strong and representative of the crop as a whole. The flowering phenology of the three types was followed for three seasons (1992–1994). Flowering sequences for wild, Glen Moy and Glen Prosen raspberry were determined by counting the number of flowers in the 1m areas at roughly 3-5 day intervals as they were either a) coming into flower, b) in full bloom or c) passing out of flower. This was repeated throughout the flowering season on four representative 1m plots throughout the beginning, peak and end of the flowering period.

2.2.2. Flower morphology

The morphological developmental stages of raspberry flowers were observed in the field during the season 1992. Ten flowers at least were marked immediately after opening in the two sites. I observed and recorded morphological changes in the flowering stages during the flowering life span.

2.2.3 Anther dehiscence and pollen availability

In the early morning ten flowers which were just opening were marked, and at intervals of 2 hours through the day the anther dehiscence of the flowers was observed using a field lens. I examined the number of opened anthers at each sampling time. Flowers were observed from 0800h to 1800h at both sites.

Isolated observations of pollen availability in different stages of raspberry flowers were made in the field during a day. At least 10 buds were loosely bagged with muslin, on each raspberry type. Once the flowers had opened the bags were removed and a soft paintbrush was passed firmly three times over the anthers. This was repeated with flowers of different ages. The brush loads were then discharged into Fuchsin-glycerine jelly on glass slides as described by Beattie (1971a), thus fixing and staining the pollen grains. The preparations were protected with a coverslip and examined by light microscopy in the laboratory later.

In order to assess the number of pollen grains available for the pollinator in the field, the same technique was used by sampling the pollen from at least 10 unprotected flowers throughout the day. The samplings were in the early morning (0800), mid day (1200) and evening (1830).

2.3. Nectar production.

2.3.1. Effects of flower ageing (longevity)

Large unopened flower buds were chosen and bagged with muslin cloth to exclude pollinators or nectar thieves. Immediately after the blossoms opened, each blossom was labelled with a tag to ensure repetitive sampling of the same individuals throughout the experiment. Nectar samples were taken at both sites at 0800; the flower was rebagged and nectar extracted again from the same flower 24 h later. Sampling was repeated at 24 h intervals till the flowers began withering. The nectar was withdrawn with 1µl or 5ml micro capillary tubes (Camlab UK). Nectar volumes were determined by measuring the length of nectar columns in the capillary tube. Where volume per flower exceeded 5µl, repeat samplings were taken, and the total volume recorded from the total length of capillary tube filled. Sugar content was determined immediately after

sampling in the field by using a pocket refractometer (Bellingham & Stanley Ltd.), giving % sugar (weight/weight).

The daily nectar production assessments were repeated twice for each variety with different numbers of flowers on different dates; for example the sampling of Glen Moy was on 28 May (n = 20) and 4 June (n = 17), for Glen Prosen on 17 June (n = 16) and 25 June (n = 13) and for wild raspberry on 15 June (n = 10) and 22 June (n = 12) and all the samplings were taken in the season 1992.

2.3.2. The effect of repeated nectar removal from flowers on nectar secretion.

In order to assess the effect of repeated nectar removal throughout the day on nectar secretion, big flower buds were numbered and bagged by muslin on each raspberry type. Immediately after the flower opening, the nectar was extracted from halfe of them with 1µl or 5µl micro-capillary tubes, and sugar contents were determined, as described above, and the flower rebagged.

The flowers were repeatedly sampled at 2 hour intervals throughout the day. Sampling started in the early morning at 0800 h and continued till 1800 h in the evening (BST). The another halfe of the opening flowers were sampled once at the end of the same day at 1800h. This experiment was repeated twice during the season.

2.3.3. Nectar volume and sugar concentration of raspberry throughout the day with insect visitation.

In order to assess the availability of nectar in the field throughout the day with insect visitation, floral nectar rewards from the three varieties were followed in unprotected flowers throughout the day. Nectar was withdrawn

from the flower in 1µl or 5µl micro capillary tube and dispensed into the pocket refractometers to record sugar concentration. At least 10 flowers were sampled at intervals of 2 hs through the day. The visitors were observed within the raspberry plants on the same days, to determine the response of bees to daily changes in nectar availability. A measurement of the visitor's activity was obtained by assessing the average number of visitors during a 30 min. period at 2 hour intervals through the day, just after the nectar sampling had taken place, in four representative 1m plots.

2.4. Insect diversity.

To determine the diversity of insects on raspberry flowers, I moved throughout the sites for at least 2h every 3-4 days during the season 1992, 1993 and 1994, capturing as many bees as possible with either an insect net or jar. Insects were killed with ethyl acetate and taken to the laboratory and pinned. By using the taxonomic references available (Alford 1975; Willmer 1985; Gilbert 1986; Prys-Jones & Corbet 1991), most of the collection was identified to the species and some insects to genus. The specimens were checked and verified by comparing with insect collections in Edinburgh at the Royal Museum of Scotland, Chamber Street.

2.5. Determining the seasonal abundance of visitors.

The changes in abundance of each visitor over the season were compared with the changes in weather conditions and flowering number on each variety during the season. A measure of visitor abundance was obtained from censuses made every 3-6 days at each site. The census consisted of observing 1m lengths of the raspberry plant for 30 mins. Honey bees and bumble bees seen on flowers were easily identified (conspicuous differences in thoracic and abdominal marking made field identification of bumble bees possible). The other visitors were identified to genus, because of the difficulty of identifying to

species in the field. To control for any diurnal changes in visitor activity, censuses were always conducted at the same time of day (between 1000h and 1200h BST). The abundances have been standardised to the average number of bees seen per 30 min in 1m. The measurements of abundance were done on the same days as measurements of flowering phenology (see 2.2.1.). Environmental factors were monitored throughout the season as described later (see 2.8).

2.6. Foraging behaviour.

2.6.1. Bee behaviour

The behaviour of the visitors (nectar or pollen gathering, or both) throughout all visits observed in the two seasons 1992 - 1993 was recorded; these behaviours were very easily distinguished on the open raspberry flowers, and confirmed for pollen gatherers from the subsequent behaviour of the bee in transferring gathered pollen to the corbiculae. Also the preferences of the visitor for the different ages of flowers were observed and recorded.

2.6.2. The timing of foraging bouts

To estimate the timing of foraging in the raspberry plants, I used Pleasant's (1981) method. Two stopwatches were used, one to record the total period of observation and the other to record handling time. Handling time was equal to the time spent by the bee on flowers. Travel time (either between flowers or between plants) was equal to the difference between total time and summed handling time. Bees were observed for a minimum of 20 visits per foraging bout. Data on 200 - 800 flower visits by bees were obtained for each raspberry variety.

2.6.3. The foraging rate

In order to estimate the foraging rate of bees on the raspberry flowers, the number of flowers visited by one bee during 1 min was estimated at several times during the flowering period of each variety.

2.6.4. Pollen load

2.6.4.1. Insect borne pollen

The quantity of pollen carriage on bee's bodies was counted from freshly captured insects which were foraging on the raspberry flowers by using a hand net. Insects were killed with ethyl acetate, and the rear legs plus pollen basket were removed as this pollen is not available for pollination (but see Parker 1981), thus avoiding contamination of the body pollen. Each bee was put individually in a labelled vial and shaken vigorously with 5ml distilled water, and then removed to ethanol (70%) for preservation. The pollen grains in solution were kept on dry ice, before return to the laboratory for centrifugation and counting with a haemocytometer under a light microscope (Krause & Wilson 1981). Bees were also checked for any persistently adhering grains. Pollen grains were identified as *Rubus* or otherwise, and the percentage of *Rubus*-type pollen scored in each sample. Without complex SEM techniques it is impossible to be certain which type of raspberry pollen was present, or even to rule out the presence of other closely related rosaceous pollen sources, (though there were none of similar phenology close to the field).

2.6.4.2. Pollen on stigmas

Pollen transfer to stigmas by bees was assessed by using the method described by Yeboah Gyan and Woodell (1987). Large flower buds were labelled and covered with muslin bags. As soon as the buds opened the bags were removed. Immediately after the first insect visited the flower, its identity was noted and the flower was collected. The styles were carefully picked from the flowers with fine forceps, and stained immediately with Fuchsin-glycerine jelly as described by Beattie (1971a). The number of pollen grains per stigma was counted later in the laboratory under a light microscope, using 20 individual stigmas per flower. At the same time, styles were sampled from uncovered flowers which opened sychronously with the covered flowers, but which were

not visited by insects, and these were examined under the microscope as a control.

2.7. Gene flow

2.7.1. Pollinator directionality

2.7.1.1. Landing point and movements between flowers in the same plant.

I studied whether foraging bees on Glen Moy and Glen Prosen flowers showed any preferences for landing on any particular part of the bush, and their subsequent pattern of movement between individual flowers in the same plant. Observations were made on *Apis mellifera* and *Bombus* spp., and the bush was divided into three sectors: 1. lower, 2. middle, 3. top. The bees landing on these sectors were recorded, and the movements of the bees between the flowers of the different sectors were also recorded. The observations started at 0900 hour and ended at 1400 hour on different days throughout the flowering season 1994.

2.7.1.2. Inter floral movements

Bombus spp., *Andrena* spp. and *Apis mellifera* were the main foragers on Glen Moy and Glen Prosen. Individual pollinators were observed for at least two consecutive interplant moves so that a directional relationship could be established between each flight and the previous one. The first move of bees will be referred to as the base and the second one as the response. Each flight was scored for direction, choosing among the alternatives: north, north-east, east, south-east, south, south-west, west, north-west.

2.7.1.3. Movements between plots

To determine the pollen movements between different varieties, a field plan was designed as in Fig 2.2. Glen Clova was in the centre of the design (the donor) surrounded by Glen Lyon (the recipient) in the four different directions (South, North, East, West). To score the bee's movement in all directions, successive movements of bumble and honey bees from donor to recipient were

recorded by standing for 1 hour in each corner of the central block. I followed individual foraging pollinators from the time they left the central area until they reached the recipient plant in the different directions. I recorded all the movements to the different recipients, over several complete days.

2.7.2. Pollen dispersal

In order to monitor the actual pollen dispersal between the raspberry varieties two experiments were conducted on the SCRI field plan described above (2.7.1.)

1. Observation of whether the bees can move the dyes (pollen mimic) within the raspberry population without regarding the particular bee species responsible for the movements.

Coloured dye powder was carefully placed on the pollen of newly dehiscing anthers in all the open donor flowers (Glen Clova), before dawn when pollinators were first active. The behaviour of pollinators visiting dyed and undyed flowers was observed to confirm that this was not affected by the presence of the dye. At the end of the day, the pollen movements were measured by picking 50 flowers of the recipient plant at different distances (5, 10, 15, 45, 75, 120 meters "north- south" and 5, 10 , 15, 25, 45, 65 meters "west-east") in the different directions. I examined the stigmas by light microscopy in order to determine the number of dye-containing flowers at different distances.

2. To determine whether *Apis mellifera* or *Bombus* spp were responsible for movement of the "pollen grains" between the donor and recipient.

200 donor flowers were dyed with blue colour dye and covered with muslin. Then many *Apis mellifera* were captured from the field and placed in the covered flowers for 10 min. and released. The same procedures were followed with *Bombus* spp. on the same day with orange coloured dye. Dyed

flowers were collected to prevent cross-contamination. Later on in the day the pollen movements were measured as described above. Both experiments were repeated several times, but only twice successfully, during the flowering season.

2.8. Weather records

Environmental factors were monitored throughout each flowering season, including air temperature and relative humidity near the blossoms, using a hand-held Vaisala HMI 31 probe.

All nectar measurements and insect visits were related to macro- and micro-climatic conditions in the crop. A weather station (Squirred weather station Grant Instruments. UK), within the main site at SCRI, recorded the following: wind speed and direction, solar radiation, rainfall, ground temperature, air temperature between rows and at two heights (60 cm and 120 cm) within the crop canopy, and relative humidity between rows and at 60 cm within the canopy.

For site 2 (Cameron Reservoir), and at the gene flow site at SCRI, localised measurements of temperature and relative humidity were taken very close to the flowers being sampled or observed, using the Vaisala probe as above.

2.9 Statistical analysis

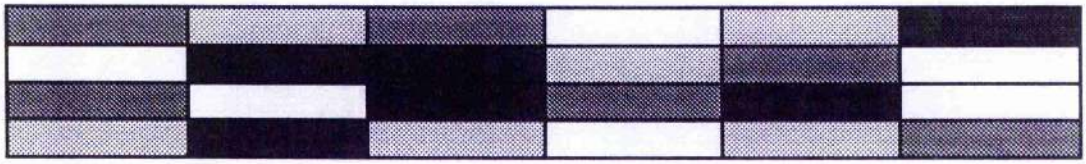
Statistical analyses were performed on PC using minitab Vr. 9.2 win. Data are expressed as mean ± SE. The difference between the means of two groups was tested with the Student's t-test; 95% confidence intervals (CI) were computed using standard parametric methods. Some data were subjected to analysis of variance (ANOVA and ANCOVA) were carried out using a

generalised linear model. and regression analyses. A P value of <0.05 was accepted as showing statistical significance.

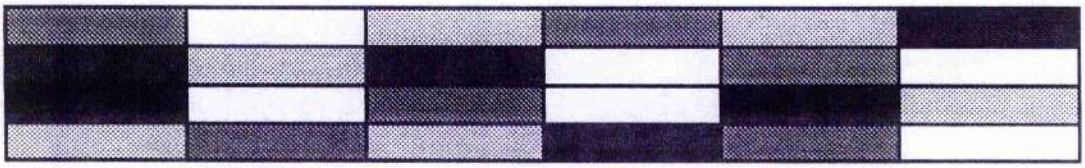
All the graphs were generated on a Macintosh using Cricket graph Ver. III

Fig 2.1 The distributions of different cultivated raspberry varieties on Site 1, SCRI. (Each sub-plot contains 5 rows of canes running north-south, spaced 2m apart.)

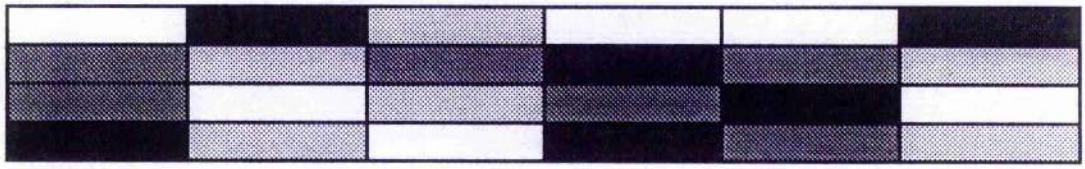
Block I



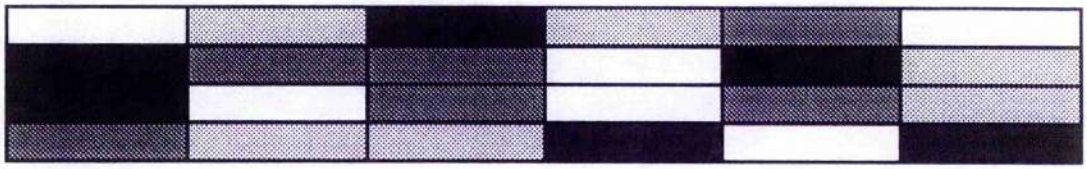
Block II



Block III (Row-Thined- every 2nd row removed)



Block IV



Glen Moy

Glen Prosen

Glen Clova (not used)

Malling Orion (not used)

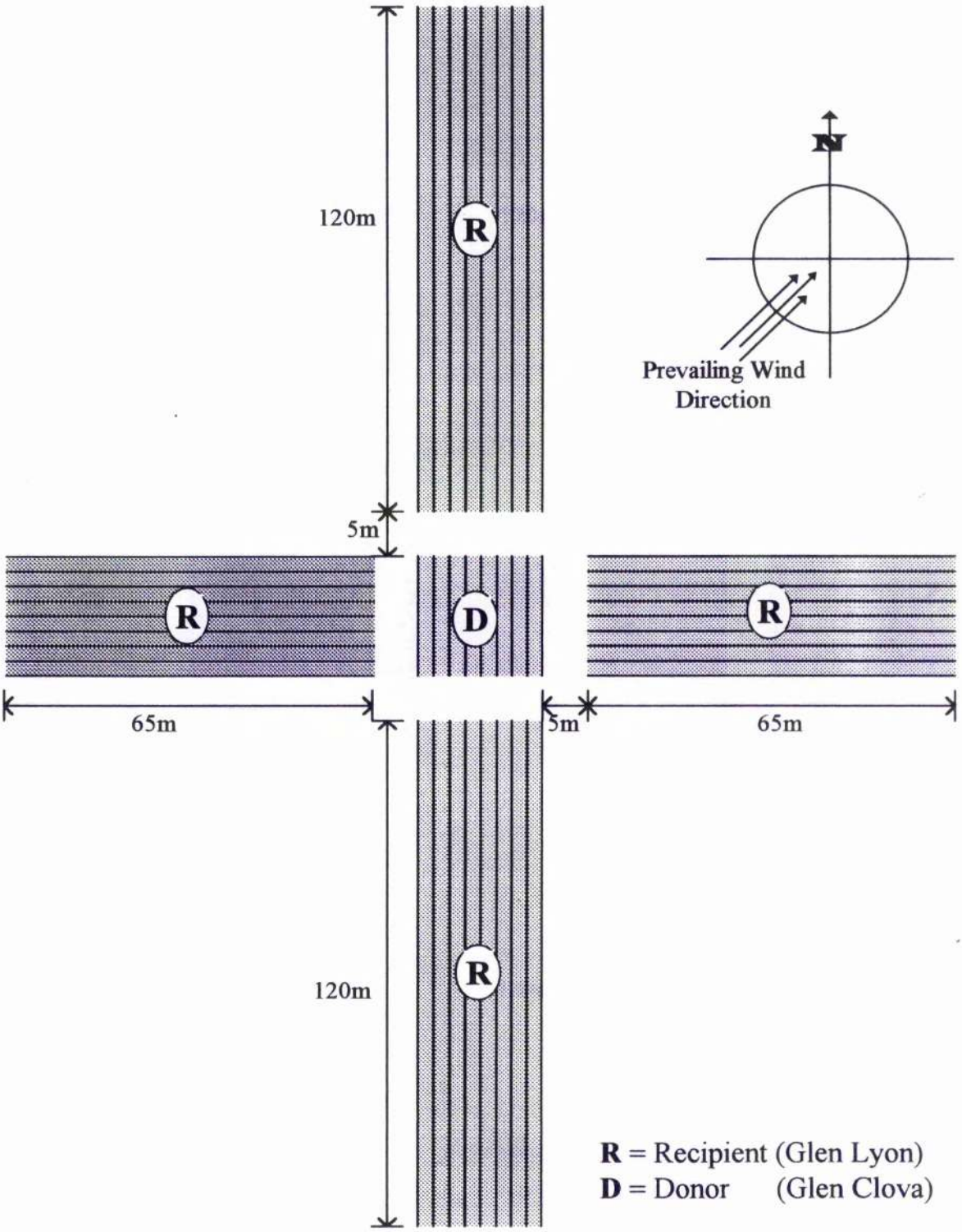


Fig. 2.2. The SCRI field desiged to monitor the gene flow between the raspbery varieties, showing the direction of planted rows of raspberry canes.

Chapter 3

Floral Structure and Anthesis

3.1. Introduction

An understanding of the broad features of the floral structure of each raspberry cultivar is essential for the correct interpretation of their pollination biology. In this chapter, the flower morphology, phenology and anther dehiscence and pollen availability of each cultivar are discussed. These observations, although apart from the main subject of this work, are the necessary foundation for the next chapters.

Glen Moy and Glen Prosen (both bred and released at SCRI in 1981) are spine-free cultivars. Glen Moy was bred as a large-fruited and high flavour variety, and flowers somewhat earlier, while Glen Prosen was specifically bred as a late fruiting cultivar (Jennings 1988).

Flowering time is a trait which could be critical to plant success through its effect on reproductive processes such as pollination and timing of seed dispersal. Optimal flowering time may be a trade-off between a variety of selective factors including pollinator availability (Waser 1978).

Flower longevity, the length of time an individual flower remains open in the field with functional stigma and stamens, is important in understanding pollination ecology as a dynamic process. The longevity of a flower determines, given a certain level of activity by pollinators, the probability and the number of times that the flower will be visited (Primack 1985).

Pollen is a rich source of food, especially of protein. Bees therefore use great quantities of pollen for their larvae, and when functioning as an attractant, in general it is well exposed and available (Faegri & van der Pijl 1979). Pollen is presented at certain periods only, and its obvious presentation must be synchronised with nectar presentation where nectar is the chief attractant.

Percival (1955) showed that in some flowers anthers dehisce at certain periods only, and she has distinguished between different types from ''early morning'' to ''night'' crops. The anthers within a single blossom may dehisce simultaneously or within a few hours, or they may expose themselves gradually over a long period. Microclimate influences the timing of flower-opening, anther extrusion and dehiscence (Seaton & Kremer 1938; Corbet 1990), and anther dehiscence probably depends on loss of water from the endothecium by evaporation (Schmid & Alpert 1977). Jones and Newell (1946) showed that the timing of pollen release in several species of grass depended on the weather; in some species it could be delayed by low temperature or high relative humidity. Percival (1950) also investigated temperature, and showed that it is more important than relative humidity in limiting anther dehiscence.

Little work has been done on raspberry varieties, describing in detail anther dehiscence or pollen available (see Willmer, Bataw and Hughes 1994). My interest in this chapter centres on a series of related questions:

1. How variable is flowering phenology from year to year ?
2. Are there any differences in anther dehiscence or pollen availability between the raspberry cultivars?
3. What are the effects of microclimatic factors on anther dehiscence throughout a day?

3.2. Flower morphology

A flower of wild and cultivated raspberry has five sepals and petals; the petals are small and white in colour, and the sepals persist until the fruit is ripe. The stamens arise in two crowded whorls in numbers ranging from 60 to 90. The styles arise spirally on the terminal part of the receptacle, whose size and shape consequently determines the size and shape of the fruit. The nectar glands are located between the receptacle and stamens (Fig 3.1)

The stages of floral morphology and pollen reward status of Glen Moy, Glen Prosen and wild raspberry were similar. Petals started to open on a bud at any time through a day; they were recorded as ''young flowers'' rather than as buds from the moment that one of the incurving petals was free at the tip of the bud, potentially allowing access to a persistent insect visitor. The "young flower" stage was taken as continuing up to the time that petals were reflexed beyond an angle of 90° to each other, with stamens visibly dehiscent and bearing the white/cream pollen, and stigmas also a pale cream in colour. "Medium-aged" flowers were those with petals reflexed out to the horizontal position 180°, with pollen mostly shed and some anthers withering and darkened, and with stigmas a dark cream in colour. "Old flowers" had petals reflexed beyond the horizontal, or already being shed, and had both anthers and stigmas darkened and beginning to shrivel. Where any particular flower showed an aberrant combination of characters the status of the stigmas was taken as conclusive.

Observations revealed that floral morphologies of similar aged cultivated and wild raspberry were essentially the same. Differences between types, however, occurred in the disc size (corolla width) of the flowers. Glen Moy had larger flowers (disc diameter 7.99 ± 0.54 mm, n = 50), Glen Prosen was smaller (6.32 ± 0.53 mm, n = 50) and wild raspberry smallest (5.76 ± 0.43 mm, n = 50); analysis of variance (one-way) showed highly significant differences between the flower disc diameter, within the three cultivars (Table 3.1). Glen Moy, with the ''showiest'' flowers, might therefore be expected to be the most attractive to visitors at long range.

Nectar in raspberry flowers is secreted abundantly, by a fleshy ring on the receptacle internal to the stamens. Nectar is, therefore, only partially concealed after anthesis so that even short-tongued insects can easily reach the

nectar, and this character could explain why the raspberry flowers attract a diverse group of insects.

3.3. Flowering phenology

The flowering season in 1992 started earlier in Glen Moy than in either Glen Prosen and wild raspberry; flowering of Glen Moy was initiated in late May, peaked in early June and ended in early July. In Glen Prosen, flowering coincided with the peak bloom period of wild raspberry; flowering was initiated in early June, peaked in mid June, and ended in late July (fig 3.2a).

In 1993, the flowering season started earlier in Glen Moy than in either Glen Prosen or wild raspberry (fig 3.2b) and somewhat earlier than 1992. Flowering of Glen Moy was initiated in mid May, peaked in mid June and ended in mid July. In Glen Prosen, flowering again coincided with the peak bloom period of wild raspberry; flowering was initiated in early June, peaked in mid June, and ended in mid July.

Flowering season in 1994 also started earlier in Glen Moy than Glen Prosen or wild raspberry; flowering started in the beginning of June, peaked in mid June and ended in early July. In Glen Prosen and wild raspberry flowering started a few days later than Glen Moy; the wild plant peaked in mid June and ended in early July, while Glen Prosen peaked a few days after the middle of June and ended in the middle of July.

Glen Moy had far more flowers at its peak (means of 129 flowers per meter in 1992, 107 flowers per meter in 1993 and 50 flowers per meter in 1994) than Glen Prosen (61 flowers per meter in 1992, 60 flowers in 1993 and 36 flowers in 1994) and wild raspberry (84 flowers per meter in 1992, 75 flowers per meter in 1993 and 34 flowers per meter in 1994) (Fig 3.2). The flowering observations revealed that Glen Moy produced more flowers than Glen Prosen

or wild raspberry in all three flowering seasons especially in the flowering season 1992, while the flowering season 1994 had less flowers compared with the two previous seasons; that could be as a result of the raspberry plant age, or the weather conditions.

It is clear from the results that the flowering time in 1993 was shifted about a week later than 1992, and the season in 1994 was also shifted a week later than 1993. Also the number of flowers per meter was decreased in all the three varieties of raspberry plants in that season.

Table 3.2 shows that in the beginning weeks of flowering Glen Moy provided the visitors with more flowers than Glen Prosen and wild raspberry. In terms of numbers, at equivalent stages of the season there was some variation in flower number per meter in the first week of flowering; Glen Moy produced 12.4 flowers and Glen Prosen and wild produced 10.8 and 8.3 flowers.

The duration of blooming by individual cultivars was also variable between cultivars but consistent between years, ranging from about 44 days in Glen Prosen, to 39 and 37 days in Glen Moy and wild raspberry respectively.

3.4. Anther dehiscence and pollen availability.

3.4.1. Anther dehiscence

Darrow (1920) showed that the stigmas of red raspberry *Rubus idaeus* are receptive long before the anthers open. Therefore pollen production from anthers is the main constraint on the timing of pollination, and my studies concentrated on this aspect. In my experiments the flowers which were chosen were just opened, and the examinations were conducted during sunny days.

The start of dehiscence had already occurred when the flowers were opening, around 0800h. When the weather was warm (ambient temperature 11.9 °C and relative humidity 75%) about 10% of anthers were dehisced in Glen Moy flowers and the mean percentage of anther dehiscence increased during a day to reach the peak of nearly 98% at 1800h (ambient temperature 17.9 °C and relative humidity 57 %) (Fig 3.3). In Glen Prosen flowers about 19% of anther dehiscence had occurred at 0800h (ambient temperature 11.9 °C and relative humidity 93 %) and this increased to the peak at 1900h (ambient temperature 18.3 °C and relative humidity 63 %) (Fig 3.4). In wild raspberry flowers at 0800 h the mean percentage of anther dehiscence was 11.3 % (ambient temperature 12 °C and relative humidity 76 %) and again this increased during the day to peak at 2000h (ambient temperature 17.6 °C and relative humidity 61 %) (Fig 3.5).

3.4.2. Factors affecting Anther dehiscence

My experiment was not designed to analyse the factors affecting anther dehiscence, because all data in this experiment represent the records of one day and so could not precisely judge the effect of temperature and relative humidity on anther dehiscence; but it could give some idea about the effect of these factors on the anther dehiscence.

Correlation coefficients were calculated between temperature, humidity and the percentages of anther dehiscence throughout the day, and the results are as indicated in figures 3.6 - 3.8. My examinations of anther dehiscence for Glen Moy were between 11.5 °C and 19.0 °C and 44 and 75 % Rh. For Glen Prosen all examinations were recorded between 11.9 °C and 19.0 °C and 59% and 93% Rh, and for wild raspberry between 11.9 °C and 18.0 °C and 53 % and 76 % RH.

The effects of ambient temperature and relative humidity on anther dehiscence were fairly consistent, but roughly opposite to each other. In Glen Moy, the percentage of anther dehiscence significantly increased with rise in temperature ($r = 0.9114$, $n=6$, $P< 0.05$) (Fig 3.6) and significantly decreased with relative humidity ($r = - 0.7839$, $n=6$, $P<0.05$). In Glen Prosen anther dehiscence again significantly increased when temperature increased ($r = 0.9148$, $n=6$, $P<0.05$), and decreased with relative humidity ($r = -0.8121$, $n=6$, $P<0.05$) (Fig 3.7). In the case of wild raspberry flowers (Fig 3.8) the anther dehiscence was again significantly correlated with increase in ambient temperature throughout a day ($r = 0.9535$, $n=6$, $P<0.05$), and with decrease in relative humidity ($r = - 0.7516$, $n=6$, $P<0.05$). Since regression coefficient are always high for temperature effects, it may be that temperature was the main determinant of dehiscence; though it must be remembered that temperature and humidity are themselves highly (inversely) correlated and that both are strongly dependent upon time of day.

3.4.3. Pollen availability.

Pollen available on successive days after flower opening in Glen Moy, Glen Prosen and wild raspberry was assessed. Individual flowers were functional for about 4 days, and could readily be scored into the age categories described in Table 3.3. The data show a decline in the number of pollen grains from day 1 to the last day. The number of pollen grains showed extremely significant differences between the flower ages (Table 3.4). Only on day 1 was substantial pollen available for transfer, for all raspberry cultivars. Some pollen grains could be gathered from a small proportion of older flowers, but they appeared somewhat shrivelled and stained very densely, so that it is doubtful whether they were by then suitable for germination if transferred to a stigma. All cultivars showed the same trend, a decrease in the number of pollen grains with increase in the flower age.

Table 3.4. also shows no significant variation between the three raspberry cultivars. Glen Moy, Glen Prosen and wild raspberry flowers produced the same number of pollen grains. The interactions between the three raspberry cultivars and flower ages showed no significance.

I investigated pollen availability within a day by using the techniques described in Chapter 2; pollen adhering to the paint brush was greatest at 0800h in all raspberry plant flowers, with moderate amounts still available by 1200h, and low numbers at 1800h. All cultivars studied therefore produced pollen throughout the period 0800h - 1800h, but the mean number of pollen grains available through a day differed from one cultivar to another (Table 3.5). The flowers showed differences in number of pollen grains available at the same time; for example at 0800 the range available in Glen Moy was 122 - 1601 pollen grains, in Glen Prosen it was 350 - 1040 and in wild raspberry it was 201 - 900 pollen grains per flower. Glen Moy therefore appeared to be the most variable of the cultivars.

Glen Moy showed higher numbers of pollen grains throughout the day, and wild showed consistently lower numbers than Glen Moy and Glen Prosen. This suggests that Glen Moy could provide the visitors with larger quantities of what they need, and might therefore attract more visitors.

3.5. Discussion

All the studied raspberry cultivers show very similar flower morphology, but differences occur in size, and in pollen produced throughout the day. The difference in flower disc sizes indicate that Glen Moy nectaries could be more in number or volume than in the other cultivars, and this may be one of the reasons why Glen Moy produced higher amounts of nectar than Glen Prosen and wild raspberry (chapter 4). Glen Moy produced larger flowers and

more flowers per meter, and all these factors together might act to increase its attractiveness.

Seasonal flowering in 1992, 1993 and 1994 showed that raspberries typically began flowering shortly after the first period of several consecutive days of warm weather. Flowering peaks for all species thus occurred during the first period of weather consistently suitable for insect pollinators. Several things are evident from the figures. The flowering of all cultivars stretched over two months, from the beginning of June to the end of July; during this period nearly all cultivars came into flower, and this synchronised with pollinator's activities (see chapter 5). Marked overlap of the flowering period of all three forms studied occurred during the period from early June to early July. All cultivars flowered earliest in 1993, rather later in 1992 and 1994 (see fig. 3.2). The general flowering pattern exhibited was a slow increase in floral production in the initial phase, followed by a pronounced peak and then either gradual decline or a rather sharp decline (commoner in Glen Moy and wild raspberry). The numbers of flowers per meter during the season 1994 were very low compared with the seasons 1992 and 1993, and that could be due to the weather conditions or to the age of plants.

All the cultivars provided most of their pollen grains in the first day, with decreased pollen as flower age increased. This means the young flowers could best provide the visitors with what they need, for their own food and or for their nest. In terms of pollination, the greatest chances of transfer of the pollen also would occur in the first stages of the flower's life. These studies also showed that dehiscence of individual anthers may occur at any time of the day in young flowers.

The data presented indicate that temperature is probably the most important climatological factor influencing the dehiscence of the pollen sacs of

the raspberry flowers. A steady rise in the number of stamens ripening and presenting pollen occurred with increasing temperature, though more controlled studies over more days and a greater range of temperature would be needed to confirm this.

Table 3.1. Analysis of variance showing the differences between the size of flower disc of the three raspberry cultivars

Source	DF	SS	MS	F	P
vaiety	2	134.509	67.255	263.23	0.000
Error	147	37.559	0.265		
Total	149	172.068			

Table 3.2. Mean number (± SE) of flowers produced by Glen Moy, Glen Prosen and wild raspberry/1m, and mean flowering period throughout the seasons 1992,1993 and 1994.

Raspberry plants	Beginning week	Ending week	Flowering period (days)			Mean days
			1992	1993	1994	
Glen Moy	12.4 ± 4.1	1.7 ± 1.4	37	42	39	39
Glen Prosen	10.8 ± 4.6	2.0 ± 1.4	47	41	44	44
Wild	8.3 ± 2.1	2.3 ± 0.5	41	33	37	37

Table 3.3. Pollen amounts (mean no. grains gathered by standard sampling) in raspberry flowers on successive days (resampling the same set of bagged flowers at 0800 each day)

Flower age (days)	1	2	3	4
Glen Moy (n=10)	1178.0 ± 233.5	65.0 ± 18.5	4.2 ± 1.8	0.8 ± 0.3
Glen Prosen (n=10)	830.0 ± 200.3	30.6 ± 11.5	3.6 ± 1.4	0.5 ± 0.3
Wild (n=10)	637.0 ± 164.0	38.6 ± 11.4	9.1 ± 2.7	0.6 ± 0.2

Table 3.4. Analysis of variance with repeated sampling showing the effects of flower age and different cultivars on pollen grain production and the interaction between the raspberry cultivars and flower ages

Source	DF	SS	MS	F	P
Raspberries	2	435376	217688	1.73	0.197
Plants	27	3401353	125976	0.97	0.517
Stages	3	17228454	5742818	44.23	0.000
Variety*Stages	6	1228670	204778	1.58	0.164
Error	81	10517742	129849		
Total	199	32811594			

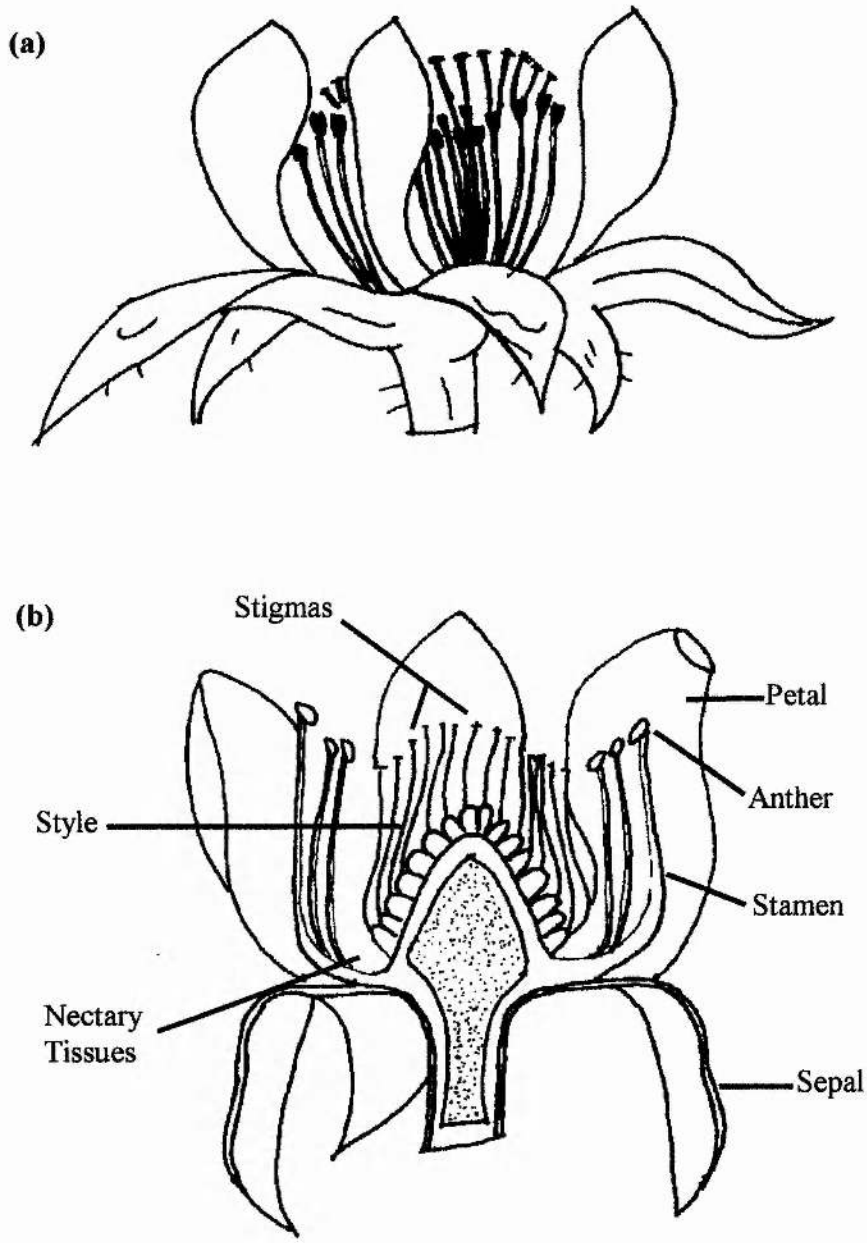


Figure 3.1 : Flowers of *Rubus idaeus*, (a) whole flower, (b) median section of flower to show the flower structure

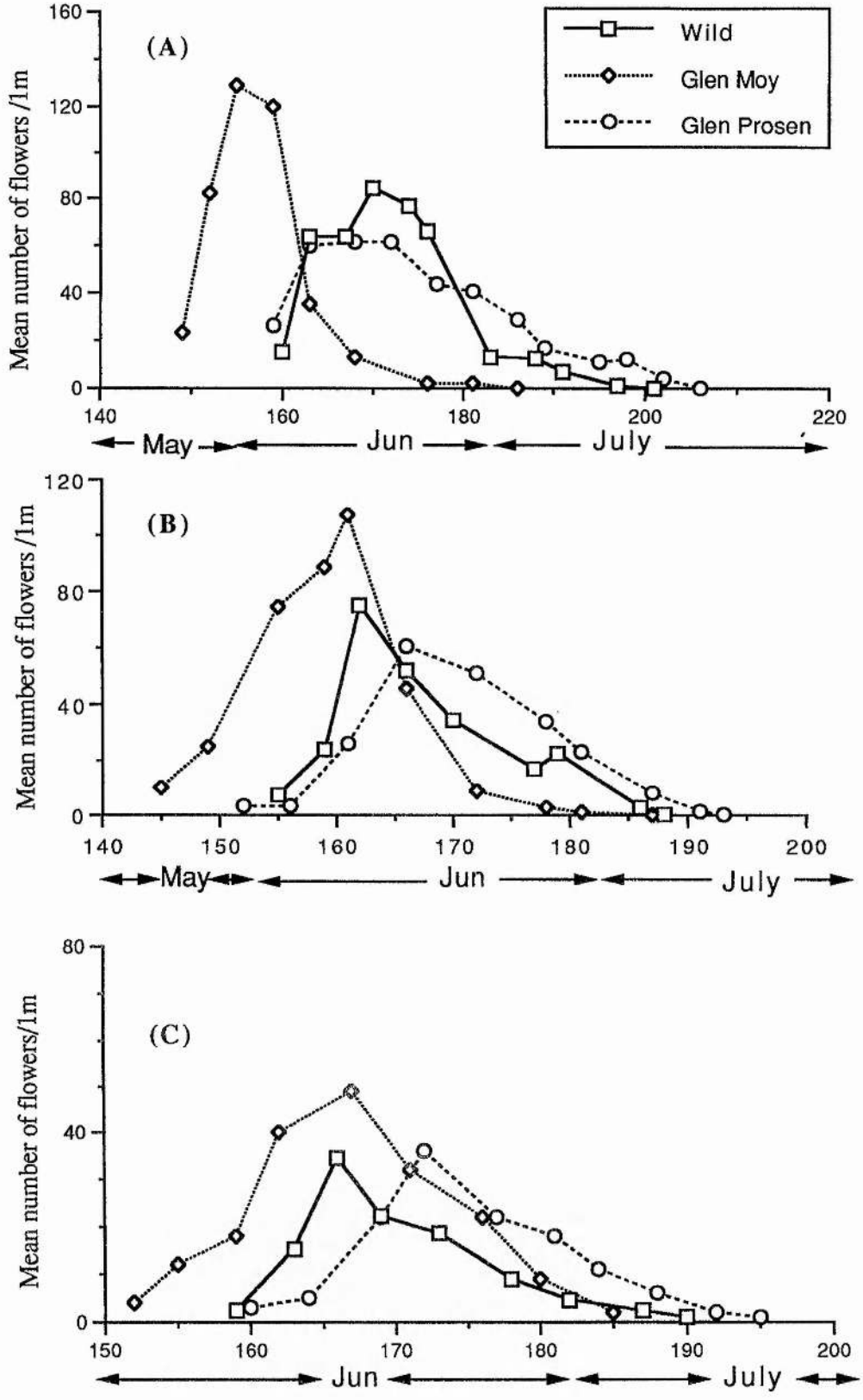


Fig. 3.2. Flowering phenology of Glen Moy, Glen Prosen and wild raspberry during the seasons 1992 (A), 1993 (B) and 1994 (C)(X axis indicates day number in a year)

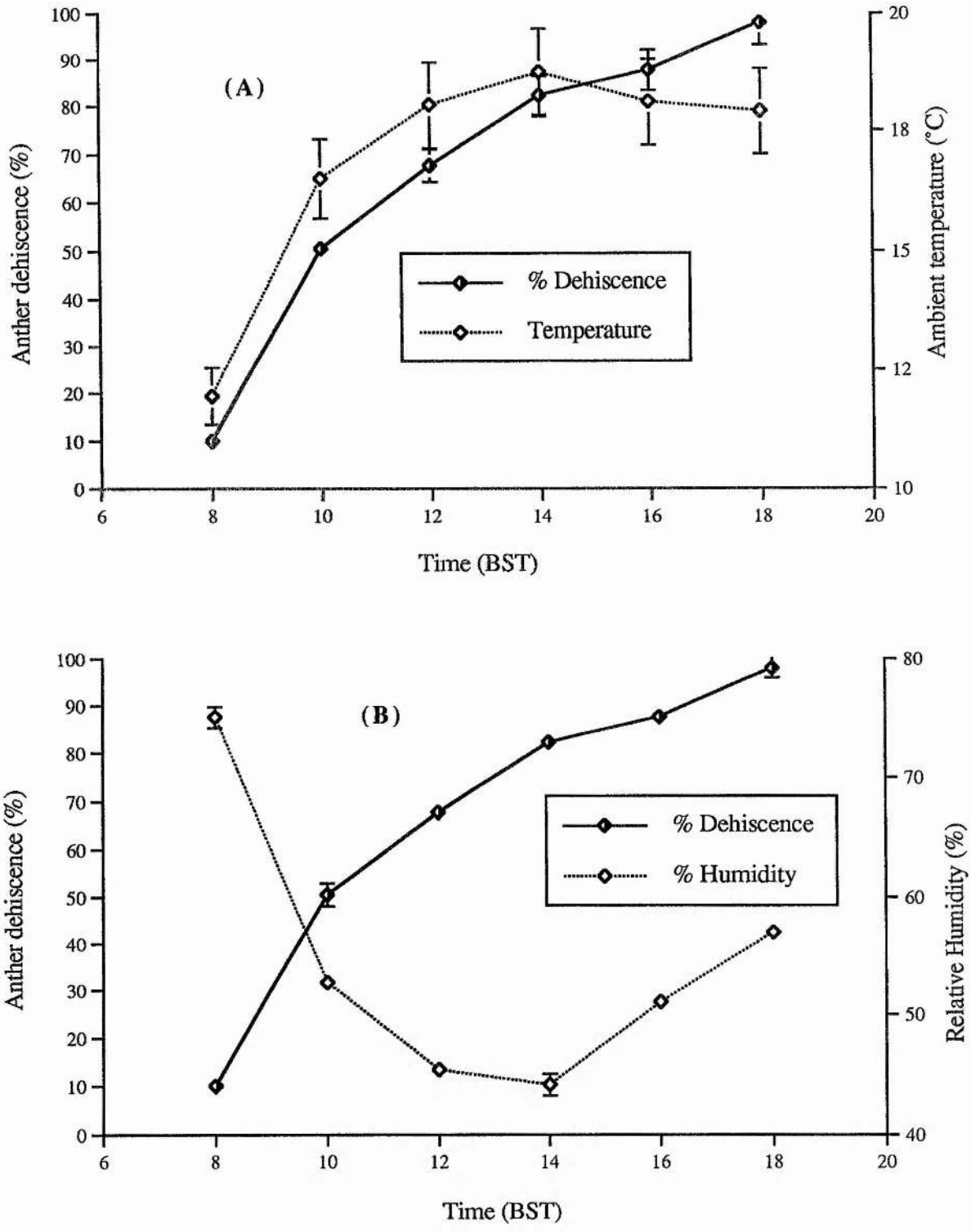


Fig. 3.3. Mean percentage of anthers dehisced in Glen Moy flowers (at least 10 flowers each sampling) through the day 1/6/1992, giving an estimate of the pollen availability to foraging visitors, with ambient temperature (a) and relative humidity (b) records. (Error bars indicate ± SE)

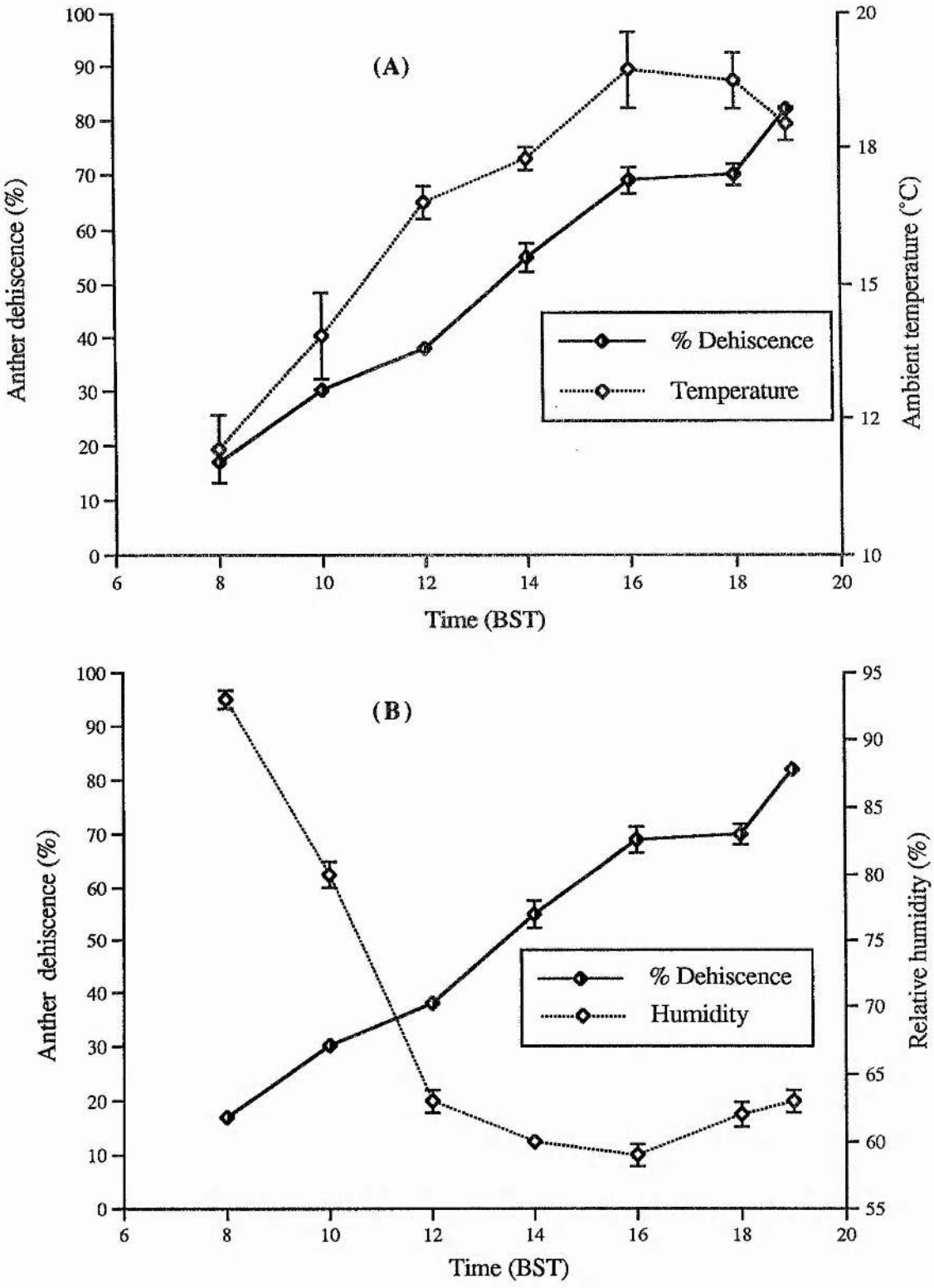


Fig. 3.4. Mean percentages of anthers dehisced in Glen Prosen flowers (at least 10 flowers each sampling) throughout the day 16.6.1992, giving an estimate of the pollen availability to foraging visitors, with ambient temperature (a) and relative humidity (b) records. (Error bars indicate ± SE)

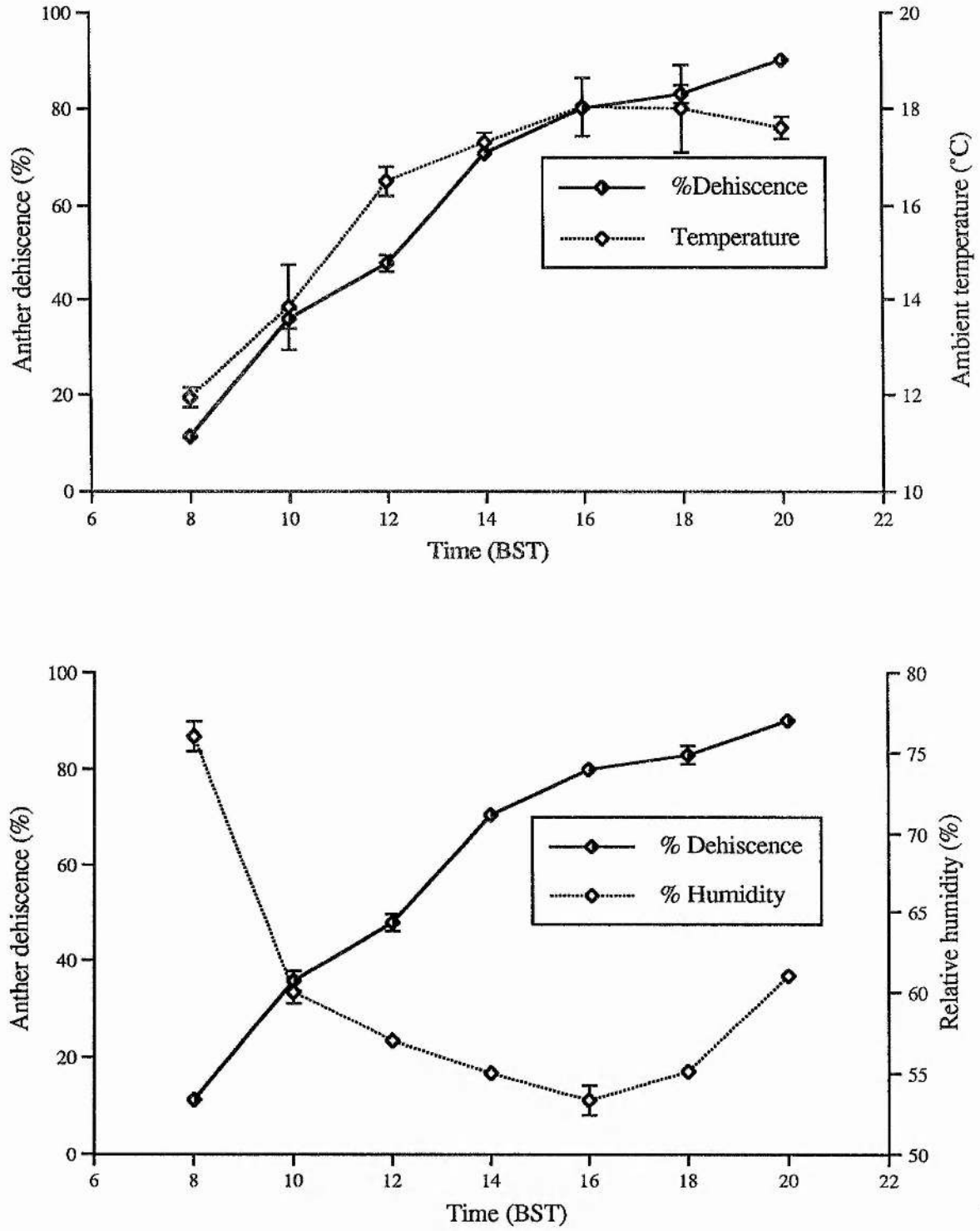


Fig. 3.5. Mean percentages of anthers dehisced in wild raspberry flowers (at least 10 flowers each sampling) through the day 1.6.1992, giving an estimate of the pollen availability to foraging visitors, with ambient temperature (a) and relative humidity (b) records. (Error bars indicate ± SE)

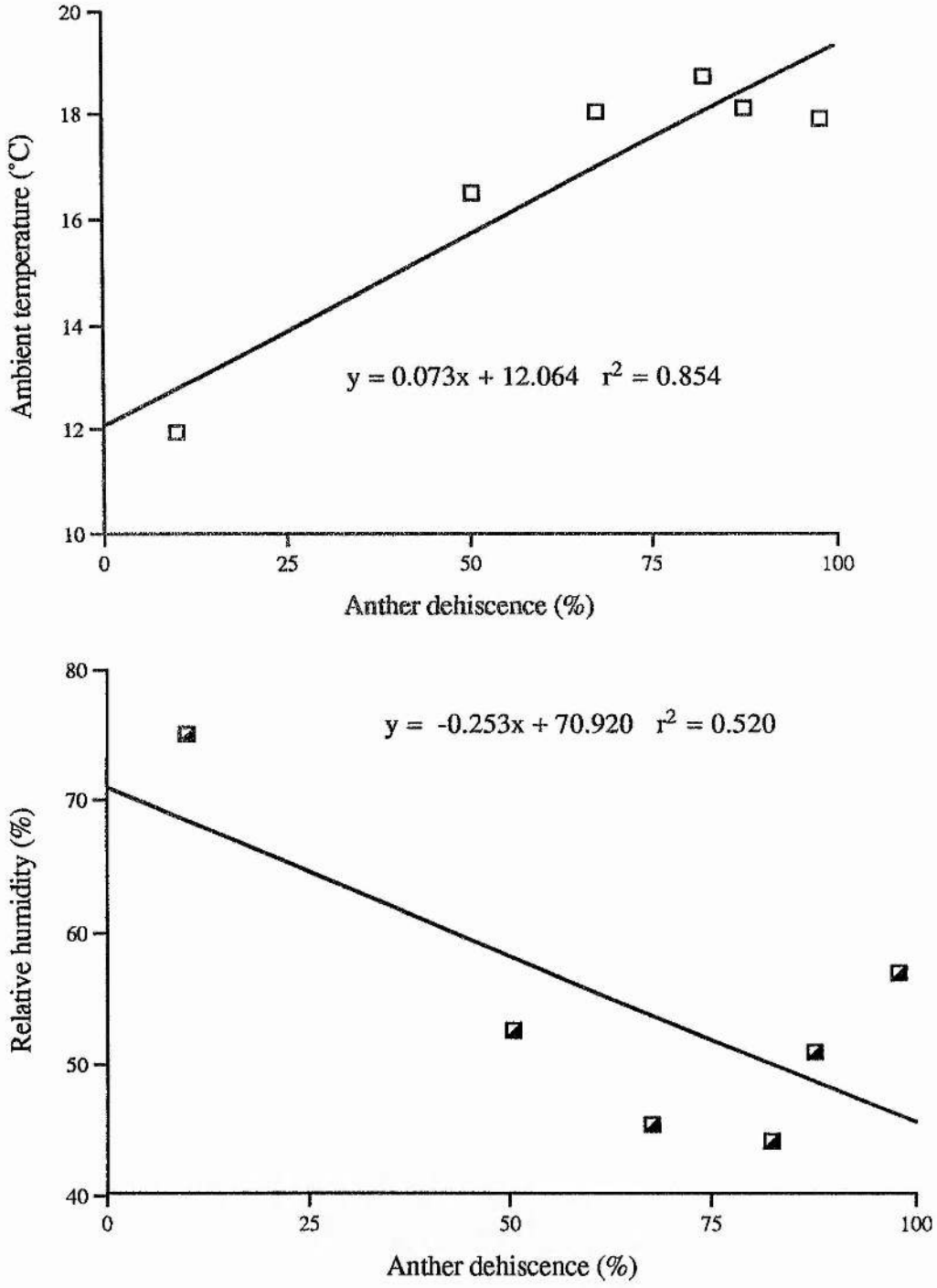


Fig. 3.6. Mean percentages of anther dehiscence of Glen Moy flowers in relation to ambient temperature (A) and relative humidity (B), throughout the day 1/6/1992 at SCRI. Calculated regression lines are shown.

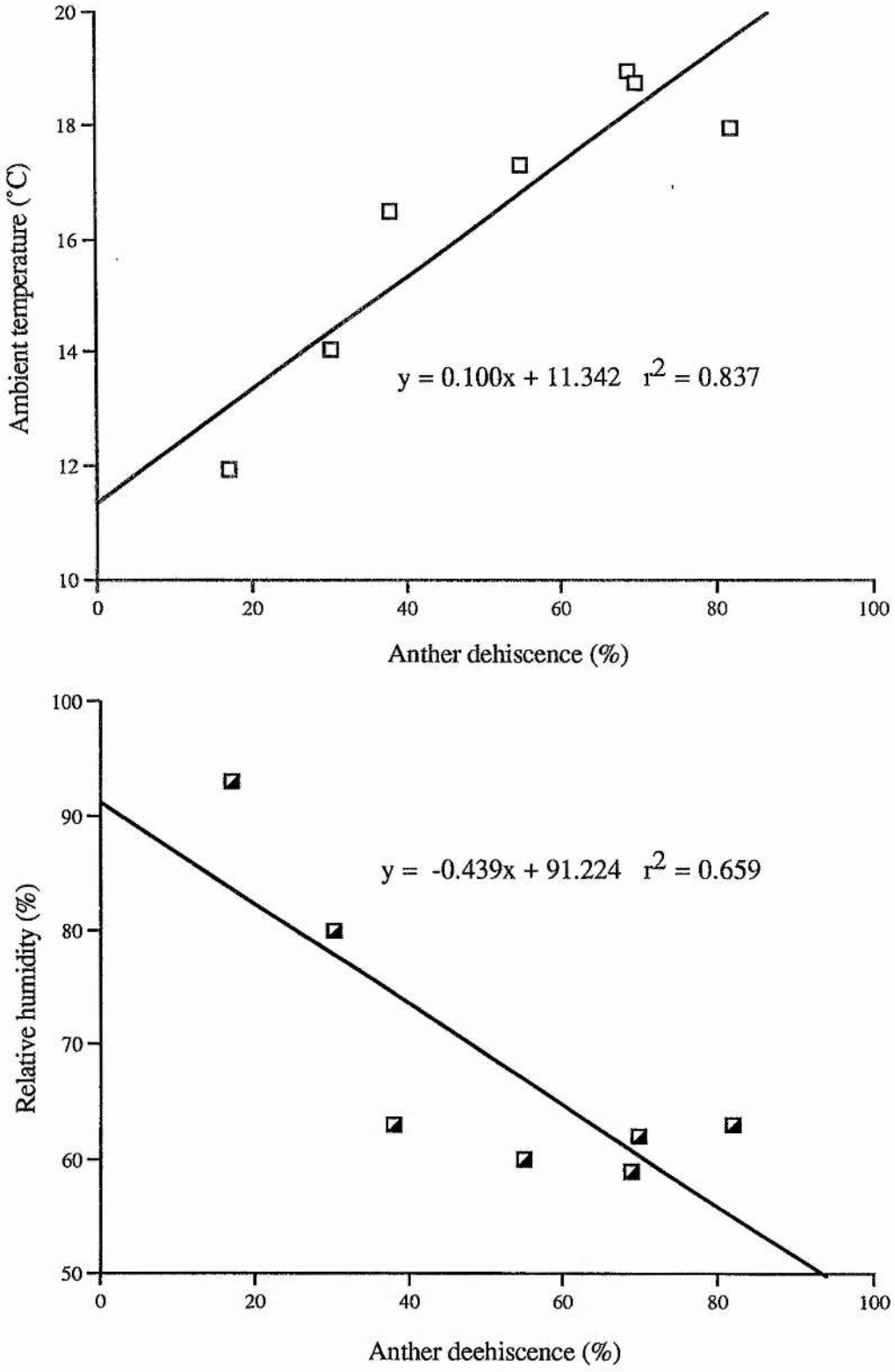


Fig. 3.7. Mean percentages of anther dehiscence of Glen Prosen flowers in relation to ambient temperature (A) and relative humidity (B)on 18.6.1992 at SCRI. Calculated regression lines are shown.

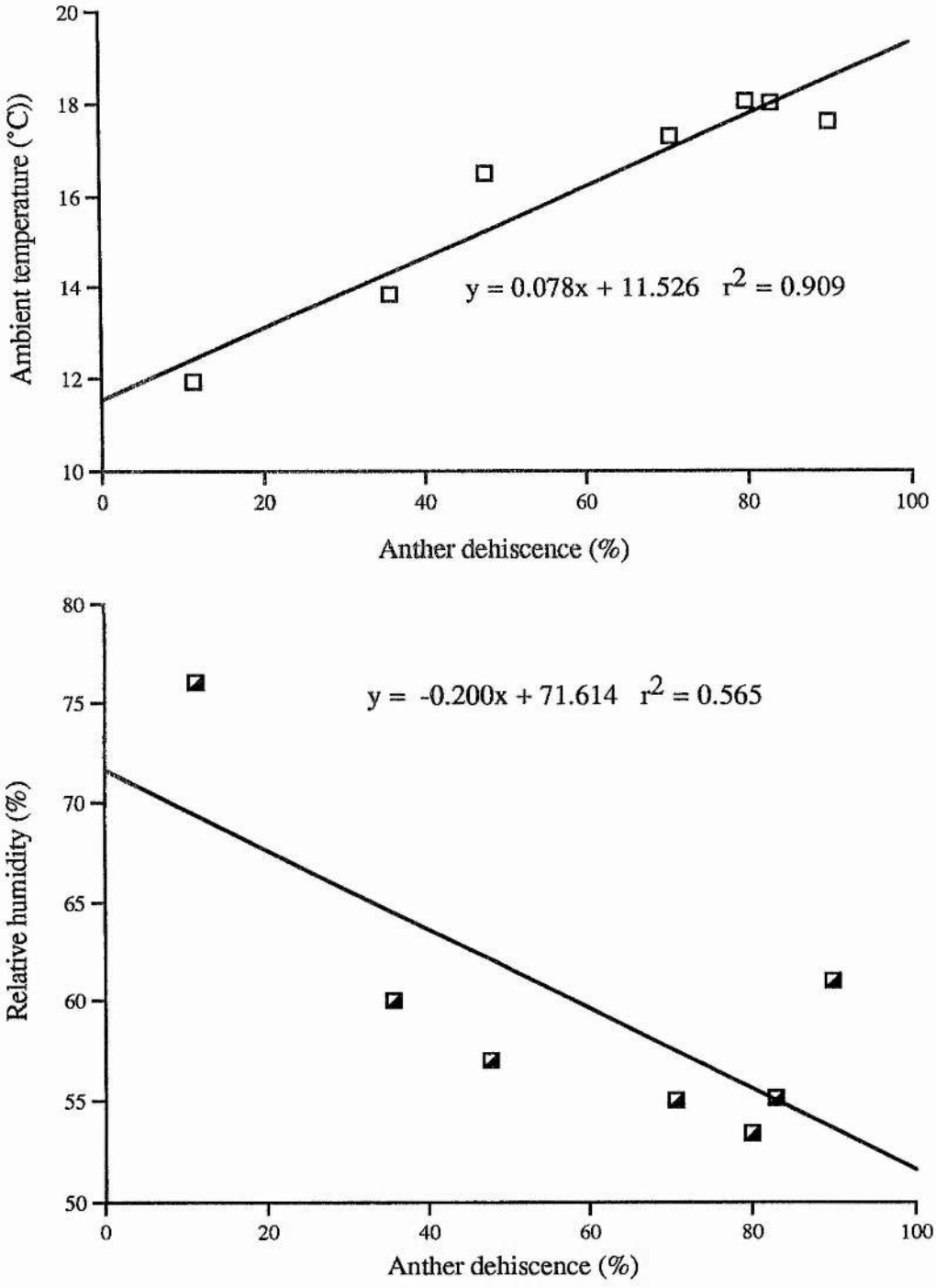


Fig. 3.8. Mean percentages of anther dehiscence of wild raspberry flowers in relation to ambient temperature (A) and relative humidity (B) on 22.6.1992 at Cameron reservoir. Calculated regressions lines are shown.

Chapter 4

Nectar production

4.1. Introduction

4.2. Ageing effects on nectar secretions.

4.3. The patterns of nectar secretion through a day.

4.4. The patterns of nectar secretion in bagged flowers through a day.

4.5. The effect of repeatedly extracting nectar from flowers.

4.6. The effects of weather conditions on nectar secretion.

4.7. Discussion

4.1. Introduction

Nectar, the primary reward in most pollination systems, is a relatively easy source of energy to study. In contrast to pollen, it has relatively simple composition, consisting mainly of the sugars sucrose, glucose and fructose (Percival 1965, Baker & Baker 1983). The study of nectar chemistry has been reviewed frequently (e.g. Baker & Baker 1975, 1983). Factors such as nectar volume and concentration, and other features that limit access to nectar, appear to be much more important than nectar chemistry in influencing flower choice by bees (Neff & Simpson 1993). Nectar production plays a vital role in pollination of flowering plants, and much work has been done on nectar production and pollinator interactions (e.g. Beutler 1953; Wykes 1953; Corbet *et al* 1979a). Here I review some of the key factors known to influence nectar characteristics.

Several studies indicate that flower age is important; its influence on nectar secretion was first recognised more than a century ago by Bonnier (1879). Collison (1973) sampled *Cucumis sativa* blossoms and found that nectar was only secreted on the first day of anthesis with none on the days thereafter. Gupta & Thakur (1987) studied *Rubus ellipticus* and revealed that nectar secretion occurred only during the first 24 h of flower opening. In other species the rate of secretion of nectar varies with the age of flower (e.g. Percival 1946, Southwick & Southwick 1983; Wood 1961). Differences in the timing of nectar production between plant species are, therefore, likely to influence greatly the relative abundance of their insect visitors through time. Very large differences have been recorded between several plant species and between genotypes in the diurnal timing and total quantity of nectar produced (e.g. Pedersen 1953; Mosquin 1971; Heinrich 1976a).

The sugar concentration of nectar is another very important property of nectar from the point of view of insect attraction. The sugar concentration of nectar and changes that take place in it during the day have been studied in a great many plants (e.g. Vansell 1934; Beutler 1953; Percival 1965; Corbet 1978a; Corbet *et al* 1979a,b; Corbet &Willmer 1981a; Willmer 1980,1983).

Many of these investigators have provided evidence to sustain the point of view that sugar concentration of nectar varies considerably among flowering species, and among individual flowers, and between times of day within one species. Such fluctuations in sugar concentration have been associated with preference for one kind of plant over another by insects (Corbet 1978b; Corbet *et al* 1979a; Rajotte & Roberts 1979; Free 1993).

Environmental factors are important in the determination of nectar volume and concentration. Kenoyer (1917) and Savos (1955) summarised the results obtained by many early workers on the effects of environmental factors on the nectar secretion and sugar concentration in different plant species. Where the study has been of a basic nature, the principles discovered should apply to secretion in general, regardless of the species of test plant used. For example Shuel (1955b) pointed out that temperature affects many plant processes which are proceeding simultaneously with nectar production. A certain threshold temperature is necessary if secretion is to occur within normal daily limits; temperature variation probably has little influence on the amount of sugar which the plant synthesises, but it has a very marked effect on the rate at which the sugar is consumed in growth, respiration and other processes. Flower development is also accelerated at high temperature. Environmental or external factors may therefore be considered as influencing nectar secretion in part through their effect on the internal factors (Shuel & Pedersen 1953) However, environmental factors also affect post-secretory equilibration of nectars (Corbet *et al* 1979b). And because the nectaries of raspberry are quite exposed, the

volume of nectar they contain is probably greatly influenced by changes in the relative humidity (Free 1993).

The timing of secretion, and the quantities of nectar offered to pollinators, are always modified by the genotype, as well as by the environmental factors that affect the flowers and the whole plant. Knowledge of the secretion of nectar in raspberry and of the factors influencing the pattern of nectar secretion and equilibration is scanty. This knowledge is important for studies of the foraging strategies of nectar-feeding animals, and for plant breeding programmes designed to improve the quantity and quality of nectar to encourage bee pollination (Walker *et al* 1974). This chapter focuses on the dynamics of nectar secretion, how the rate of secretion affects nectar concentration, and also considers the environmental factors affecting nectar production and equilibration.

4.2. Ageing effects on nectar production.

My first comparisons were of nectar production through the lifetime of the flowers of each of the cultivars, using bagged flowers to avoid visitation effects.

Nectar production by Glen Moy flowers in bagged blossoms peaked on the first day after the blossom opened and declined to zero after about 96 hrs (fig 4.1a). Nectar volume followed a similar trend during the two different sampling bouts. The first sample for each sampling bout was quantitatively different in its nectar volume; for the first sampling day (28 May 1992), the nectar volume on day 1 was 5.2 µl ± 0.9, (N = 20), and for the second sampling day (4 June 1992) was 9.1 µl ± 1.6, (N = 17). For subsequent days the differences were not significant. The sugar concentration generally decreased in the successive 24 hr samples, although in the second sampling bout the sugar concentration increased on the second day of sampling as a result of decreasing

relative humidity. As a result of these volume and concentration changes, the sugar reward per flower was always greatest for a young flower on its first day of opening and moderate on day 2; but by 3 days and 4 the flowers were fairly empty, However there was considerable variation between flowers in absolute reward.

The mean total nectar volume sampled during the first sampling bout was 12.9 µl per flower, and 19.4 µl per flower at the second sampling bout. The individual flowers of Glen Moy obviously showed a variation in nectar secretion, especially on the first day of the first sampling bout (28 May); the secretion volume varied from 15 µl to 0.5 µl, and the nectar concentration in the same bout varied from 64% in one flower, to 15% in another flower. In the second sampling bout from 4 June, the nectar volume showed differences from 18.5 µl to 0.3 ml per flower located on the same plant, and the nectar concentration varied from 69% to 5%.

Fig 4.1 (b) shows that the amounts of nectar secreted by Glen Prosen flowers again peaked in the first 24 h after the blossom opened. On 17 June 1992, the nectar volume was 4.3 µl ± 0.5, (N = 16), while on 25 June 1992, the nectar volume was 5.5 µl ± 1.0, (N = 13). In both bouts the secretion decreased to zero by about 96 hrs. The nectar concentration again showed changes from one day to another in the two different sampling bouts, due to the changes in relative humidity.

The total nectar volume produced during the first sampling was 10.8 µl per flower and was 5.3 ml for the second sampling. The individual flowers again revealed variations in nectar secretion even in the flowers on the same plant especially on the first day of sampling. For the first sampling bout, nectar secretion was 7.3 µl on one flower and 0.2 µl on other flower, while nectar concentration varied from 75% in one flower to 50% in another. In the second

sampling bout on 25 June nectar secretion was 15.7 µl and 0.3 µl in two different flowers on the same plant, and the nectar concentration was 50% and 11% respectively. The variation was thus somewhat less than for Glen Moy.

Wild raspberry flowers showed the same pattern of nectar secretion during their life span, following the same patterns as cultivated raspberry flowers in their secretion of nectar (fig 4.1c). The nectar secretion peaked on the first day of sampling in both sampling bouts. From 15 to 18 June 1992, the highest amount of nectar was secreted on the first day (8.2 µl), then declined gradually until 96 hrs when the flower showed no nectar. The total amount of nectar produced by the flower in this period was 13 µl per flower. The wild raspberry flowers again showed differences between individuals, especially on the first day of sampling. One flower produced 15.7 µl, and another on the same branch produced 0.2 µl.

For the second sampling bout, which extended from 22 to 25 June 1992, again the nectar secretion peaked on the first day of anthesis (on 22 June), and declined gradually, though the ''life span'' this time extended only to 72 hrs. The total amount of nectar produced by the wild raspberry flower in this time was 10.7 µl.

The nectar in the two different sampling bouts showed a decline in concentration (%) with increases in the flower age. The amount of sugar (mg) therefore also showed a peak on the first day of both sampling bouts. Sugar content shows a highly significant difference between the two periods ($P<0.0001$); for the first sampling period between 15 to 18 June the total amount of sugar produced by wild flowers was nearly twice the amount of nectar produced by the wild raspberry flowers between 22 to 25 June 1992.

The flowers of Glen Moy, Glen Prosen and wild flowers showed highly significant differences ($P<0.001$) between the amounts of nectar produced by the different flower stages (ages) (Table 4.1). The amount of nectar always decreased with increase in flower age; in all cases the amount of nectar produced by the flowers of the three cultivars throughout their life-span also showed highly significant differences ($P<0.001$) between sampling bouts during the flowering seasons, probably related to weather.

The pattern of nectar production varied between cultivars over the life span of the raspberry's flowers. Glen Moy produced an average 16.15 ml per flower, Glen Prosen 8.10 µl and wild raspberry 10.75 µl. Production was always maximum during the first two days of the blossom, then declined. Glen Moy produced about 44.27% of the total nectar production on the first day and about 30.65% on the second day. But Glen Prosen and wild raspberry flowers produced about 60% of the total nectar production on the first day, and 23% on the second day.

The results show that the "life span" of Glen Moy flowers range between 4 - 5 days, and for Glen Prosen and wild raspberry from 3 - 4 days. Many factors could affect the flower's life span; for example environmental conditions and the sampling date during the season could be among the reasons responsible for these differences.

The rate of secretion of sugar (mg) varies with the flower ages. This is illustrated by the curves of figure (4.1a-c) showing the amount of sugar secreted by each raspberry cultivar during the period of investigations (sampling days discussed on the figures). The data show that the major amount of sugar (mg) was secreted in the first 48 hrs of the flower's life, then the secretion fell off rapidly, but continued at a very low level right to the end of the flower's life.

4.3. The patterns of nectar secretion through a day.

In order to characterise differences in nectar production accurately among old and young flowers of the raspberries, it was first necessary to examine the diurnal pattern of production of both old and young flowers at the same time, under the same conditions. The unbagged flowers were sampled in the early morning 0800h and at 2 hours intervals throughout the day.

The diurnal pattern of nectar production of old and young flowers in all the raspberry cultivars showed the same patterns (fig 4.2a-c). The nectar production peaked in the morning at 0800 h and declined until 1600 h when the secretion ceased in old flowers, while nectar secretion continued in young flowers until 1800 hrs and then ceased (zero values after these times are not shown). The amount of nectar between the old and young flowers showed highly significant differences ($P < 0.001$). Young flowers produced more than double the nectar amount produced by old flowers in all the three cultivars, which could explain why bumble bees strongly select the young flowers (Chapter 6).

Nectar concentration (% sucrose equivalents) was dilute in the morning, and gradually increased with decrease in relative humidity; around midday the nectar became more concentrated and in the afternoon when the relative humidity increased the nectar concentration decreased and became dilute again. This phenomenon was recorded for all the studied raspberries, either old or young flowers.

Fig 4.2a-c also show milligrams of sugar in the old and young flowers of Glen Moy, Glen Prosen and wild raspberry in relation to nectar volume and concentration of each cultivar. The pattern did not vary greatly for the three cultivars, either in old or young flowers. The amount of sugar (mg) produced

by the old and young flowers peaked in the morning, with significantly less production ($P<0.001$) in the afternoons. The sugar contents were high at 0800 h but fell rapidly, thereafter remaining low. for wild raspberry some increase was measured at 1400 h, but values then fell again. Young flowers produced roughly twice the amount of sugar (mg) produced by the old ones at any sampling time through the day. The amount of sugar produced by Glen Moy and Glen Prosen young flowers showed highly significant differences ($P < 0.05$), Glen Moy young flowers producing twice as much sugar at any sampling time as Glen Prosen did. Young flowers of wild raspberry propably produce more sugar than cultivated raspberry did (similar volumes, but higher concentrations), but I cannot confirm that statistically as a result of differences in sampling days.

Since this trial involved unprotected flowers, the fall in the volume and sugar content of old and young flowers could be due to the increase of insect foraging activities.

4.4. The patterns of nectar secretion in bagged flowers through a day

In order to understand the patterns of nectar secretion through the day and the amount of nectar offered by the different cultivars to the insect visitors at different times of the day, I conducted two sampling bouts for each cultivar with bagged young flowers, so that visitors were excluded. This also allowed me to analyse the effects of temperature and relative humidity on the nectar concentration in undepleted flowers.

Fig 4.3 (a-c) shows nectar volume and nectar concentration of the flowers of bagged Glen Moy, Glen Prosen and wild raspberry respectively, in relation to temperature and relative humidity. The pattern of nectar secretion did not vary greatly for the three cultivars. Volume was always high at 0800 h but fell very rapidly thereafter, remaining low until 1600 h when the flower showed

no nectar. The nectar began to recover by 0800 h of the second day of sampling, 24 hours after the first sampling.

Individual flowers of each cultivar differed considerably in the amount of nectar they produced, especially during the earlier part of the day. At 0800 h, when nectar secretion was at a peak, as much as 15.3 µl. of nectar was collected from one flower of Glen Moy on 29.5.1992. By contrast, another flower on the same branch, under fairly similar conditions, produced 2.7 µl. Similarly at 0800 h on 4.6.1992, 21.5 µl of nectar was produced by one flower and 2.8 µl produced by another flower on the same plant.

For Glen Prosen, at 0800 h on 18.6.1992 the variation was 0.7 - 2.8 µl per flower, and on 25.6.1992 it was 1.1 - 14.0 µl per flower.

Wild raspberry flowers showed the same phenomenon of differences in the amount of nectar produced by the individual flowers on the same plant. At 0800 h on 15.6.1992 the variation was 23.0 - 3.3 µl per flower, and on 22.6.1992 it was 10.3 - 2.0 µl per flower.

The nectar concentration of the flowers of the three cultivars showed marked variations with time of day and the prevailing temperature and relative humidity (fig 4.3a-c). There was an increase in concentration during the day, as temperature increased with a corresponding decrease in the relative humidity. Nectar was dilute during the morning, became more concentrated during the day, and in the evening became dilute again as a result of increased relative humidity. This phenomenon was apparent in single flowers, and in the means of the flowers of the three cultivars. The results showed that available nectar quantity and quality is very dependent on time of day of sampling. The effects of environmental factors on nectar production will be discussed later (section 4.5).

The differences in the nectar volumes produced by the raspberry flowers, and in their nectar concentration, can be ascribed to differences in the activities of the nectaries: either differences between nectaries, or differences between flowers in one plant, or between plants.

In order to estimate the amount of nectar produced by the three raspberry cultivars and whether there were any consistent differences between the total nectar produced on the first day of anthesis, the flowers of the three cultivars were sampled once a day at different times during the season 1992. Nectar samples were from young (day 1) flowers in the early morning (0800 h) to provide an accurate estimate of nectar offered by the flowers when the pollinators first visited. Table 4.2. illustrates that a high significant difference ($P<0.05$) existed between the different cultivars in producing nectar during the first day of flowering. However this was all due to Glen Prosen. The amount of nectar produced by Glen Moy was 7.60 µl ± 0.60, (N = 76), and for wild raspberry it was 7.40 µl ± 0.87, (N = 56). Glen Moy and wild raspberry therefore showed no significant difference ($P>0.05$) in the amount of nectar produced by their flowers. However Glen Prosen flowers produced only 4.10 µl ± 0.42, (N = 51) significantly lower than the other two cultivars, making it the least rewarding of the three cultivars tested here.

4.5. The effects of repeatedly extraction on nectar secretion.

This experiment was designed to study the effect of repeated extraction of nectar on the nectar secretion process, and to test whether the repeated extraction of nectar by visitors can affect the nectar offered by the flowers for the succeeding days. Both experiments were conducted on the same days to avoid any influences of weather or other factors, and each experiment was repeated twice for each cultivar. Care was taken not to damage the flower while withdrawing nectar.

Table 4.3 shows the volume and concentration of the nectar of the three cultivars of raspberry flowers (protected bagged flowers) extracted repeatedly throughout a day and the flowers which sampling at the end of a dau 1800h The differences in amount of nectar produced between the reprated sample flowers and sampled at the end of a day shows highly significant differences ($P < 0.001$); nectar production was much lower when the flower sampled at the end of a day and also the nectar concentration became shows dilute . These results suggest that repeating nectar extraction from the flowers could affect the nectar volume and nectar concentration produced by that flower. The amount of nectar accumulated from repeated sampling flowers was much higher than flowers left undisturbed. This suggests that repeated removing of nectar from raspberry flowers could stimulate the nectary glands to produce more nectar than flowers left undisturbed.

4.6. The effects of weather conditions on nectar secretion

In order to determine the effects of prevailing microclimatic conditions on nectar production and concentration of the Glen Moy, Glen Prosen and wild raspberry flowers, nectar was sampled on different days throughout the flowering season 1993 during day- light hours, together with temperature and relative humidity records taken very close to the flowers.

Figure 4.4.(a-c) illustrates the correlation between nectar volume (µl) and nectar concentration (%) produced by Glen Moy, Glen Prosen and wild raspberry flowers and relative humidity. Though there is some indication of a stepped relationship due to many nearly empty flowers in dry weather, nectar volume showed a high correlation with relative humidity. The amount of nectar increased as relative humidity increased. Nectar concentration shows a strong negative relationship with relative humidity, with no stepped aberrations, since 'empties' were not scored for concentration.

The effects of ambient temperature on nectar volume and nectar concentration are shown in Figure 4.5a-c. Nectar volume showed a negative relationship with ambient temperature, the amount of nectar produced by the raspberry flowers increasing with decreases in ambient temperature ($P<0.05$). As expected the relationship of nectar concentration with ambient temperature showed a strong positive correlation ($P < 0.05$).

It is clear that the range of nectar concentration in raspberry cultivars varies greatly from day to day and even from hour to hour, and that such changes are directly related to the temperature and relative humidity. The volume and concentration of nectar varies as the above factors vary largely because the raspberry flowers have nectaries that are relatively unprotected.

4.7. Discussion.

Many attempts to collect nectar from the flowering buds were made, and all the three cultivars of raspberry showed no nectar available at this stage. In all raspberry cultivars the nectar secretion started immediately as the first petals began to open. Thereafter patterns of daily nectar production were quantitatively different in nectar volume and sugar concentration, in association with flower age. The age of the flower has been shown to influence nectar secretion in several plants (Butler 1945, Vansell 1934). In some plants, the old flowers secrete more nectar than the young flowers (Wood 1961), in others the reverse relationship exists (Collison 1973). In Glen Moy, Glen Prosen and wild raspberry flowers the peak nectar volume occurred on the first day of blossom, and ceased after 4 - 5 days in Glen Moy, 3 - 4 days in Glen Prosen and wild raspberry flowers. All the cultivars studied showed a consistent diel pattern of nectar secretion, with a peak in the early morning. Glen Moy secreted higher amount of nectar than Glen Prosen and wild rasperry in both bagged and in unbagged flowers. Though I did not sample the bagged and unbagged young

flowers at the same time for the three cultivars, the amount of nectar produced and nectar concentration were roughly similar, and this means that bagging with muslin does not seem to have much effect.

The same secretion patterns were found in all raspberry flowers studied, though there were substantial differences in nectar volume and concentration between the two sampling dates when trials were repeated. It is clear that available nectar is very dependent on several interrelated variables: time of day of sampling and weather in particular.

These records all show that the amount of nectar shows highly significant differences between flowers of the same plant even when they are sampled at the same time. This could explain the differences between the time spent by the different insect groups in handling nectar when they visit raspberry flowers (see chapter 6). The rate of nectar intake can be affected by choice made at the individual flower as a result of variation in the amount of nectar they contain (Hodges & Russell 1981a; Marden 1984; Galen & Plowright 1985a,b).

Great care was taken during the sampling regimes not to injure the flowers in any way. However, even in flowers that had never been subjected to any sampling before, large differences in nectar secretion between the flowers on the same branch were noticed. Various factors are known to affect nectar secretion by a plant. These include the position of a flower on the plant, the age of the flower, the age of the plant, the period in the plant's flowering season at which the flower is produced, and also edaphic and microclimatic factors (Percival 1946; Jaeger 1957; Proctor and Yeo 1973). The rate of extraction by insect visiting may also affect nectar reward (Raw 1953; Percival 1965; Free 1993). Therefore the variation in nectar secretion from flower to flower within the same raspberry cultivar could be explained as the result of a number of these factors. Furthermore because the nectaries of raspberry flowers are quite

exposed, the sugar content of their nectar was greatly influenced by changes in the prevailing temperature and relative humidity (Shuel 1954; 1955b; Corbet *et al* 1979a, 1979b).

The effects of removal of nectar from raspberry flowers were investigated by Raw (1953), who suggested that removal of nectar from flowers affects the process of secretion. The total amounts of nectar and sugar secretion by flowers whose nectar was collected more than once were higher than the amounts of nectar produced by the flowers whose nectar was collected once. My results agreed with his in the case of nectar concentration and nectar volume. He suggested that this is due to differences in osmotic relations of the nectary tissue and secreted matter.

I used unprotected flowers to explore the pattern of changes in the nectar resource available to insects in the field in both old and young flowers. Both showed the same secretion pattern throughout the sampling days. The amount of nectar and nectar concentration in all raspberry plants showed variation from hour to hour between the old and young flowers, but young flowers always offered more nectar to insect visitors than old flowers throughout the day.

Under field conditions humidity and temperature effects are hard to separate, and under experimental conditions humidity control is extremely difficult to achieve (Shuel 1955a). Because the nectaries of raspberry flowers are quite exposed, the sugar concentration showed a good correlation with both temperature and humidity. And as a result of evaporation, the sugar concentration of raspberry flowers proved highly variable through the day both in bagged and unbagged flowers. When relative humidity was high the nectar volumes of raspberry flowers in all the three raspberry cultivar were high, and nectar concentrations low.

Studies of nectar production have been done in several different ways. In every case, humidity was found to have an important influence upon the concentration of the nectar, both in bagged and unbagged flowers. It is evident, therefore, that determinations of sugar concentration of nectar of raspberry flowers are of little value unless accompanied by adequate humidity records.

Finally it should be noted that the amount of nectar and sugar offered by the raspberry flowers to insect visitors was very high compared with most temperate plants (Willmer. per. com.), where volumes per flower rarely exceed 1µl. This explains why raspberry is a very popular crop with bee-keepers.

Table 4.1. Analysis of variance showing the differences between the sampling days in nectar production, and also the effects of flower age, for Glen Moy (a), Glen Prosen (b) and wild raspberry flowers (c).

(a) Glen Moy

Source	**DF**	**SS**	**MS**	**F**	**P**
Sampling days	1	95.22	95.22	5.33	< 0.0001
Flower age	3	723.56	241.19	13.51	< 0.0001
ERROR	143	2553.71	17.86		
Total	147	3372.48			

(b) Glen Prosen

Source	**DF**	**SS**	**MS**	**F**	**P**
Sampling days	1	68.924	68.924	14.77	< 0.0001
Flower age	2	221.794	110.897	23.76	< 0.0001
ERROR	83	387.409	4.668		
Total	86	678.126			

(c) Wild raspberry

Source	**DF**	**SS**	**MS**	**F**	**P**
Sampling days	1	61.49	61.49	5.43	< 0.001
Flower age	2	478.58	239.29	20.90	< 0.0001
ERROR	83	940.27	11.33		
Total	86	1475.34			

Table 4.2. Analysis of variance showing the differences between the nectar produced by the flowers of the three raspberry cultivars when they were sampled on the first day of anthesis.

Source	DF	SS	MS	F	P
Raspberry cultivars	2	435.0	217.5	10.03	< 0.0001
ERROR	180	3904.3	21.69		
Total	182	4339.3			

Table 4.3. The effect of nectar extraction (from bagged flowers) on nectar secretion and nectar concentration. (Number in brackets indicate number of samples)

	Mean nectar volume (µl.)		**Mean nectar concentration(%)**	
Raspberry variety (n)	one sample	repeated sample	one sample	repeated sample
Glen Moy (39)	7.2	12.5	42.0	32.6
Glen Prosen (28)	4.7	8.9	44.4	25.9
Wild (27)	6.4	11.6	37.9	25.7

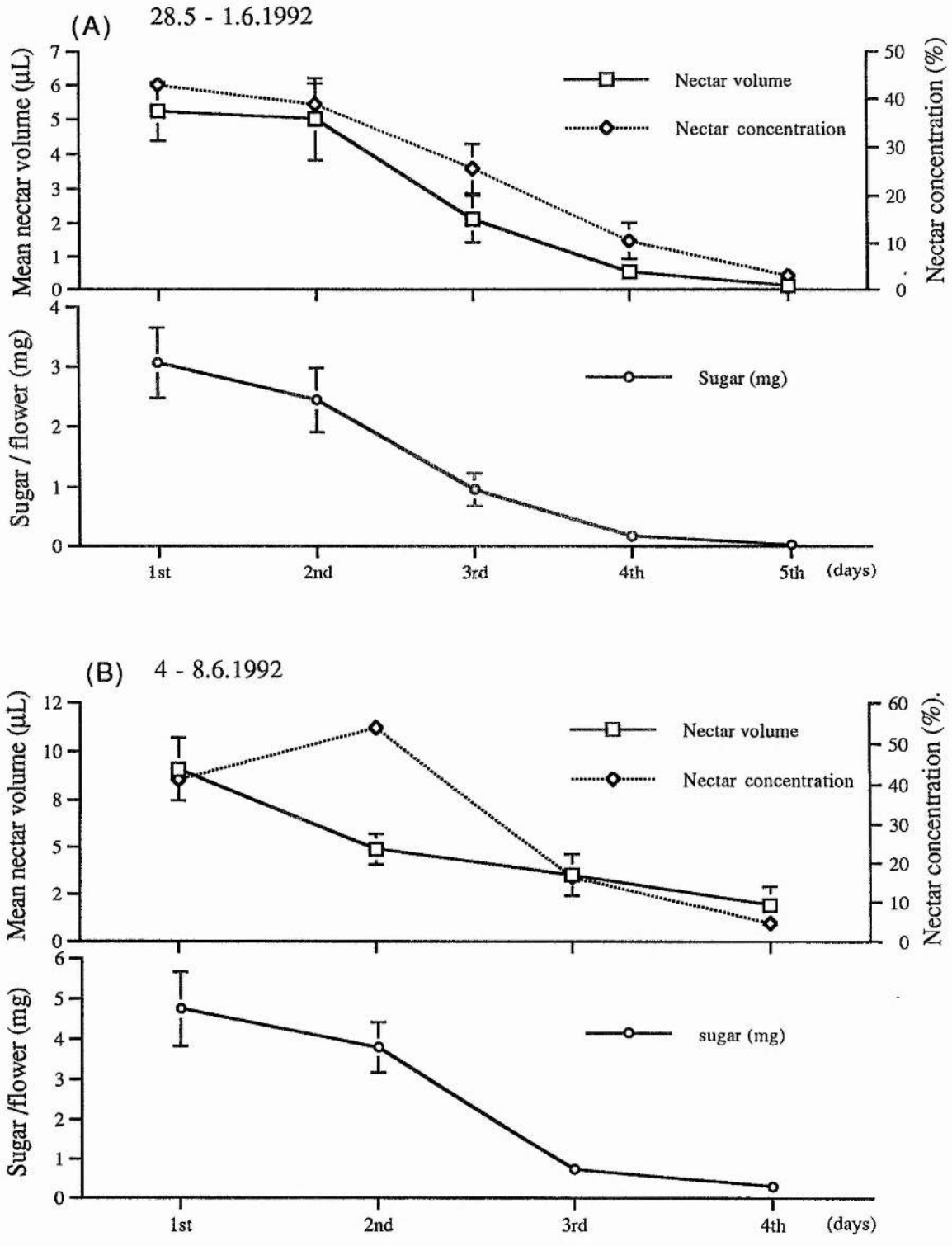


Fig. 4.1a Volume and concentration of nectar extracted from Glen Moy flowers through successive days on two different dates, at 0800 h (BST). Showing Mean ± SEM from at least 17 flowers at each sampling time. Sugar amounts (mg) are also shown.

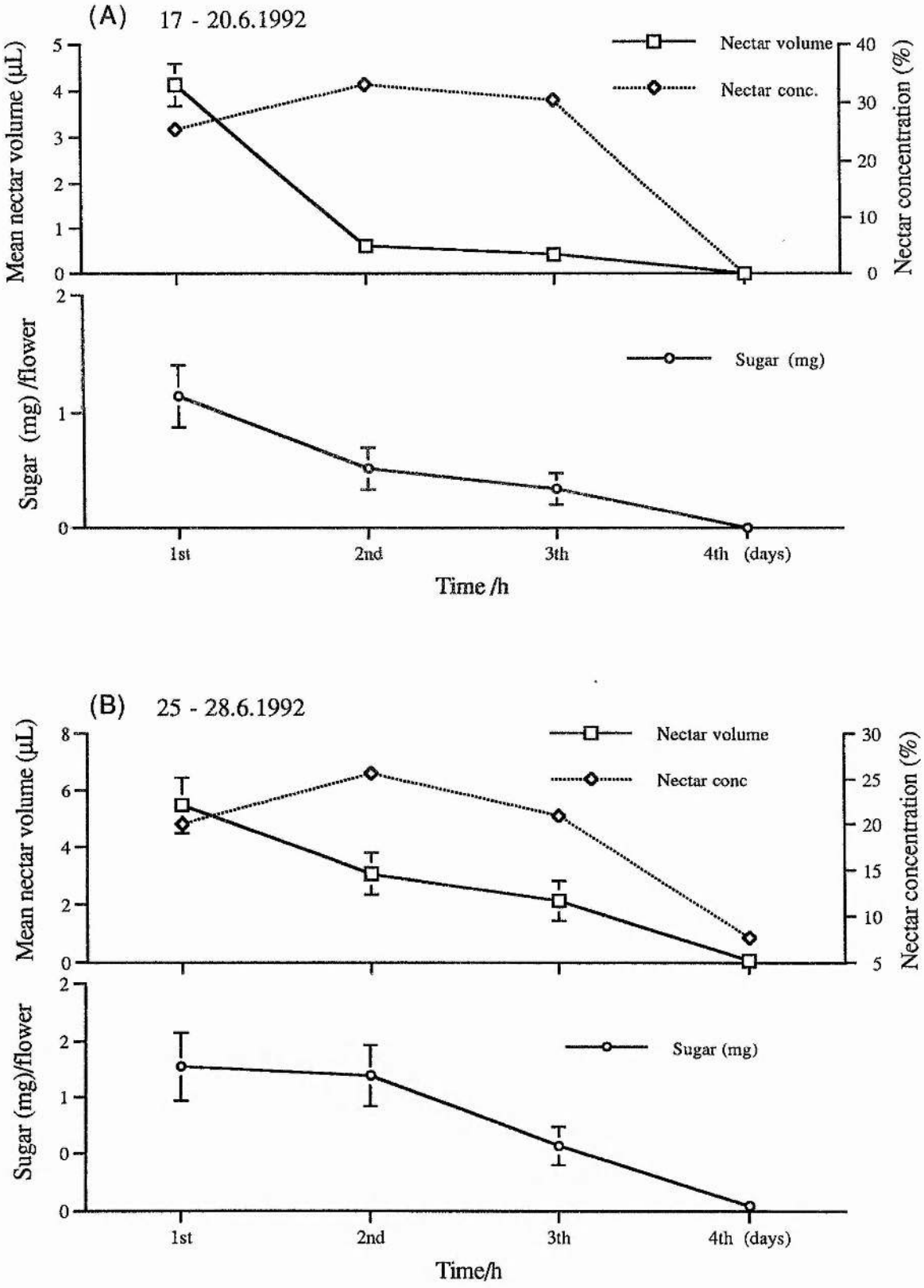


Fig. 4.1b Volume and concentration of nectar (a) extracted from Glen Prosen flowers through successive days on two different dates, at 0800 h (BST). Showing Mean ± SEM from the same16 flowers at each sampling time. Sugar amounts (mg) per flower are also shown.

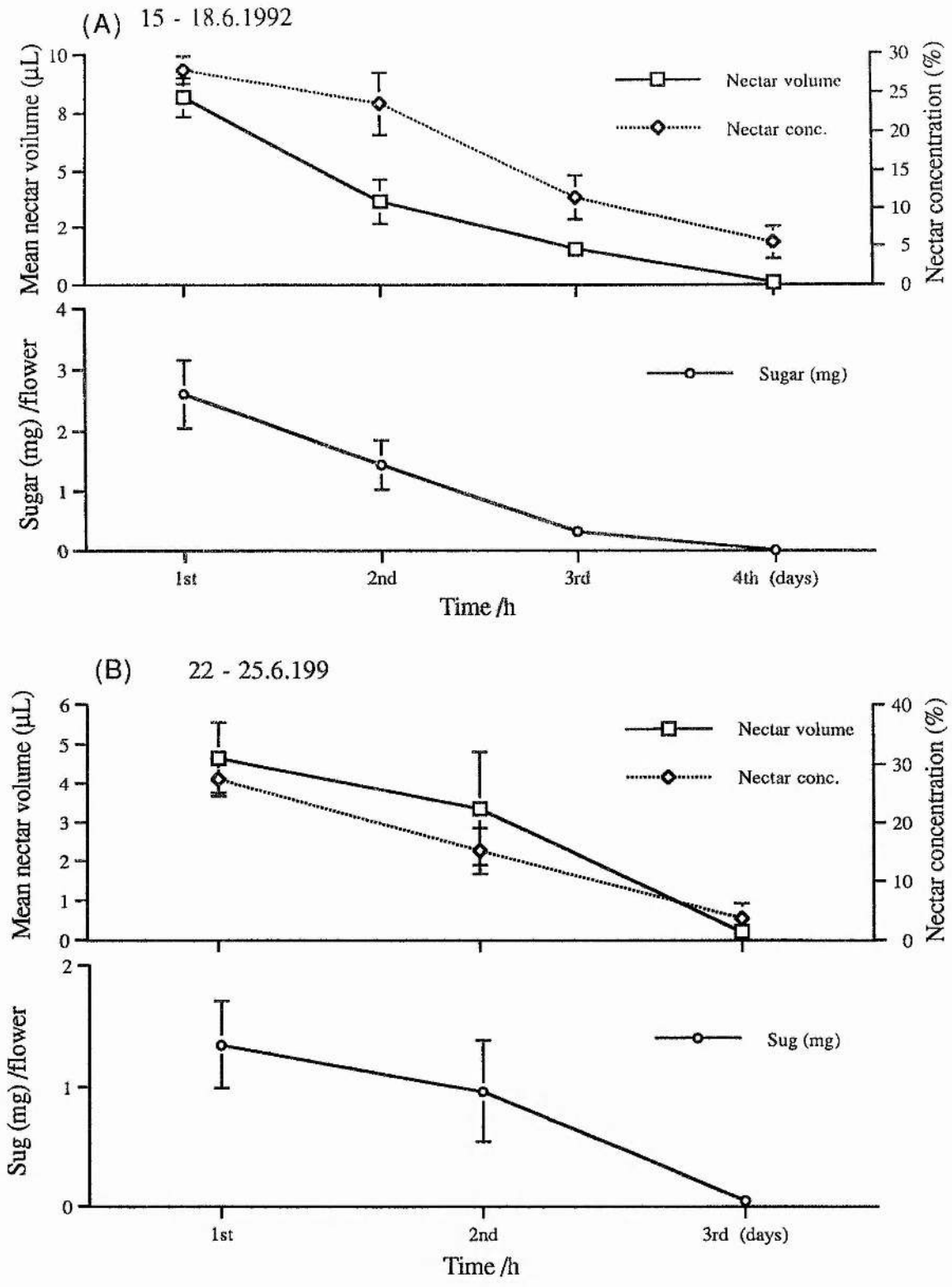


Fig. 4.1c Volume and concentration of nectar extracted from wild raspberry flowers through successive days on different dates, at 0800 BST. Showing Mean ± SEM from the same 17 flowers at each sampling time. Sugar amounts (mg) per flower are also shown.

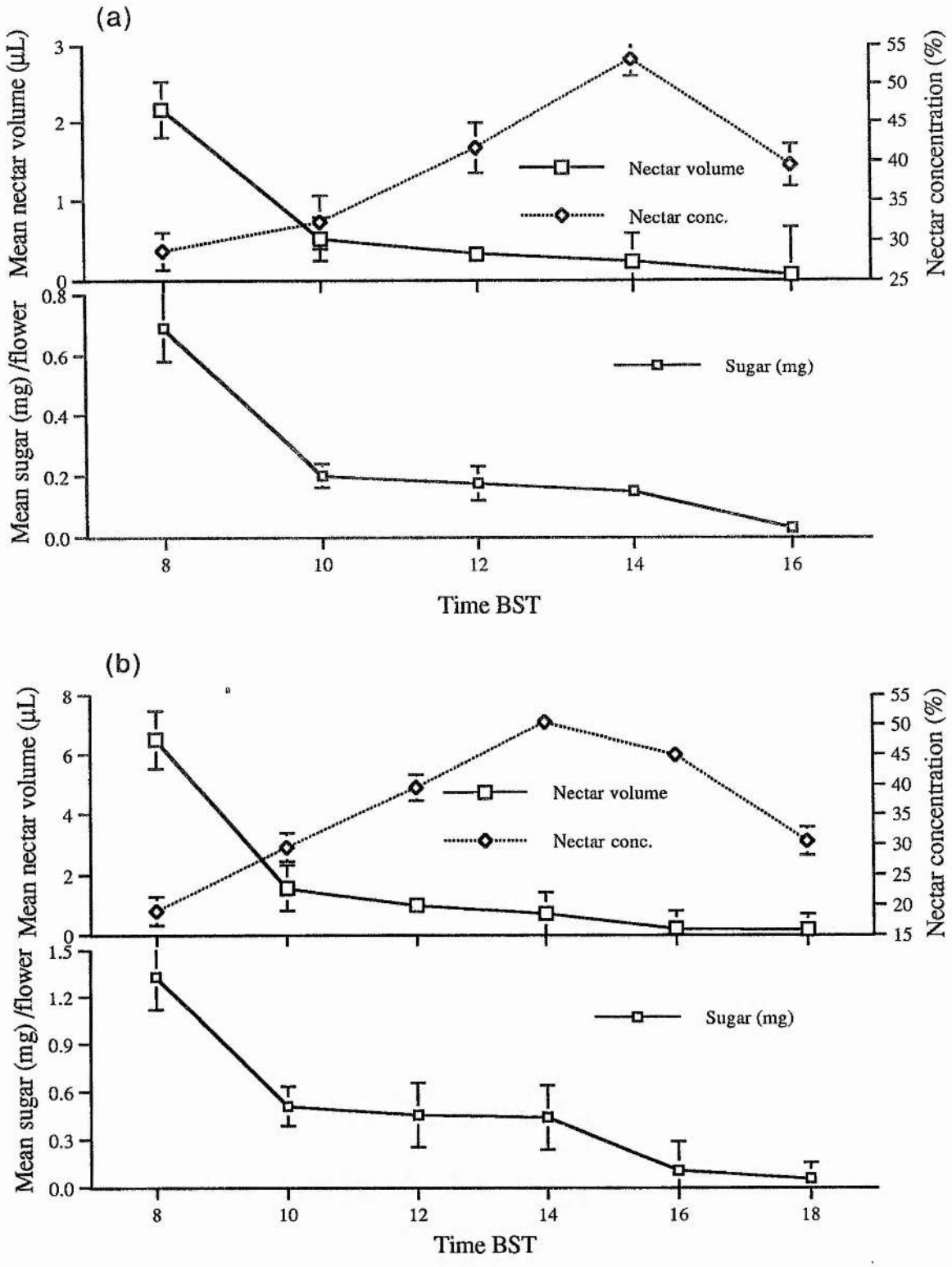


Fig. 4.2a Comparison between the volume, concentration and sugar content of Glen Moy nectar through a day, in both old (a) and young (b) flowers. The two samples were conducted on the same day (20.6.1994). Means ± SEM are shown.

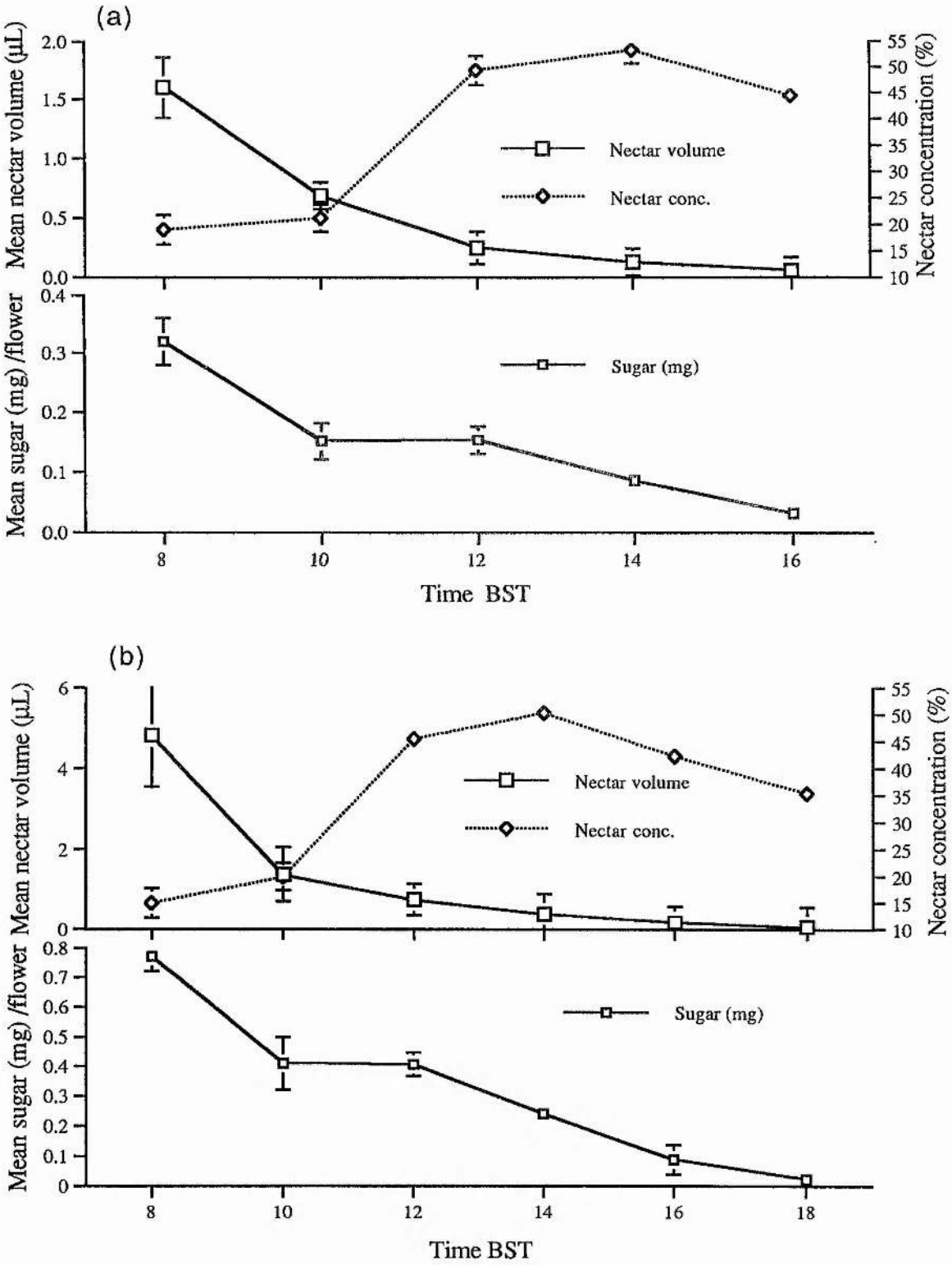


Fig. 4.2b Comparison between the volume, concentration and sugar content of Glen Prosen nectar through a day, in both old (a) and young (b) flowers. The two samples were conducted on the same day (20.6.1994). Means ± SEM are shown.

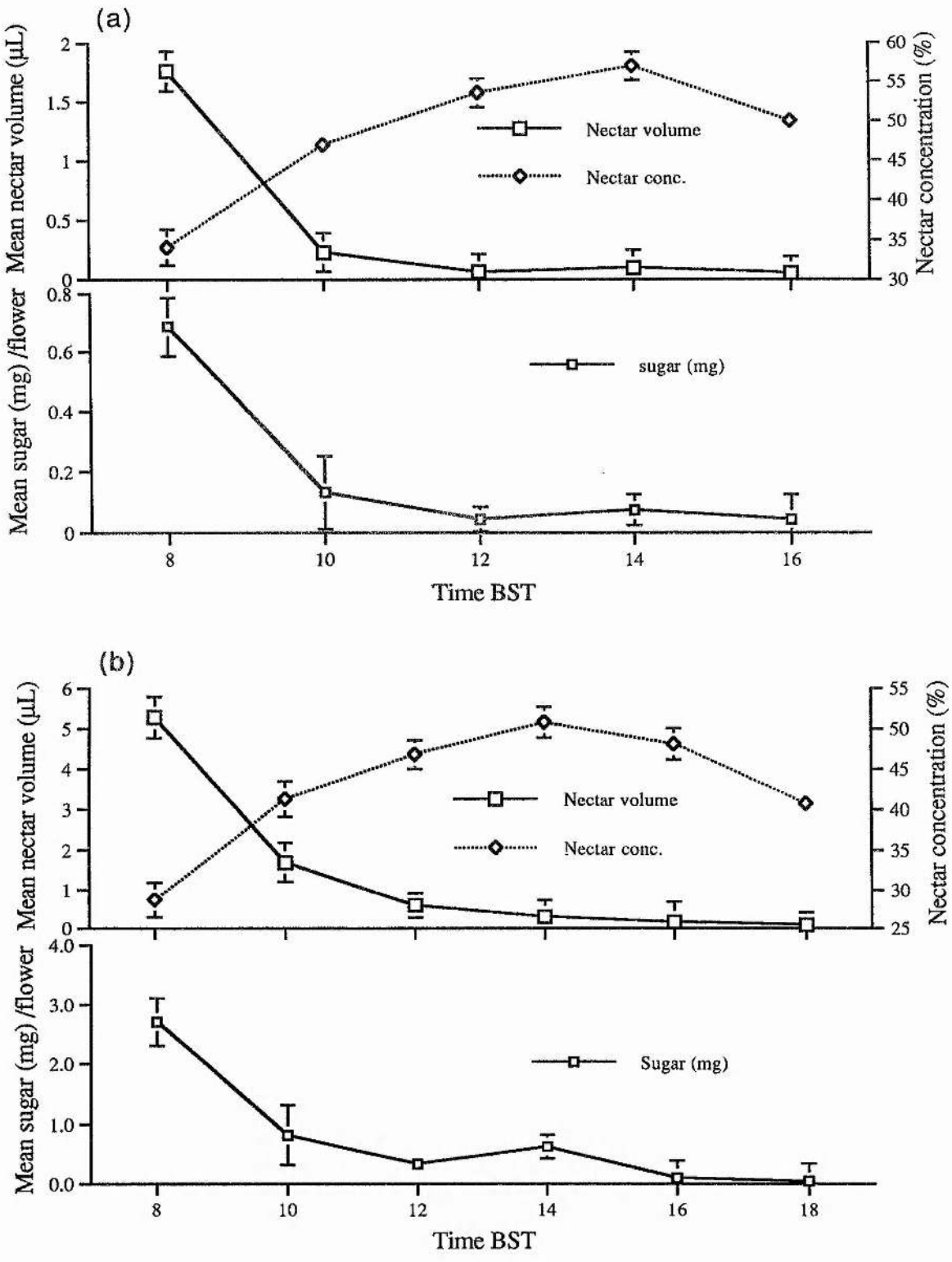


Fig. 4.2c Comparison between the volume, concentration and sugar content of wild raspberry nectar through a day, in both old (a) and young (b) flowers. The two samples were conducted on the same day (28.6.1994). Means ± SEM are shown.

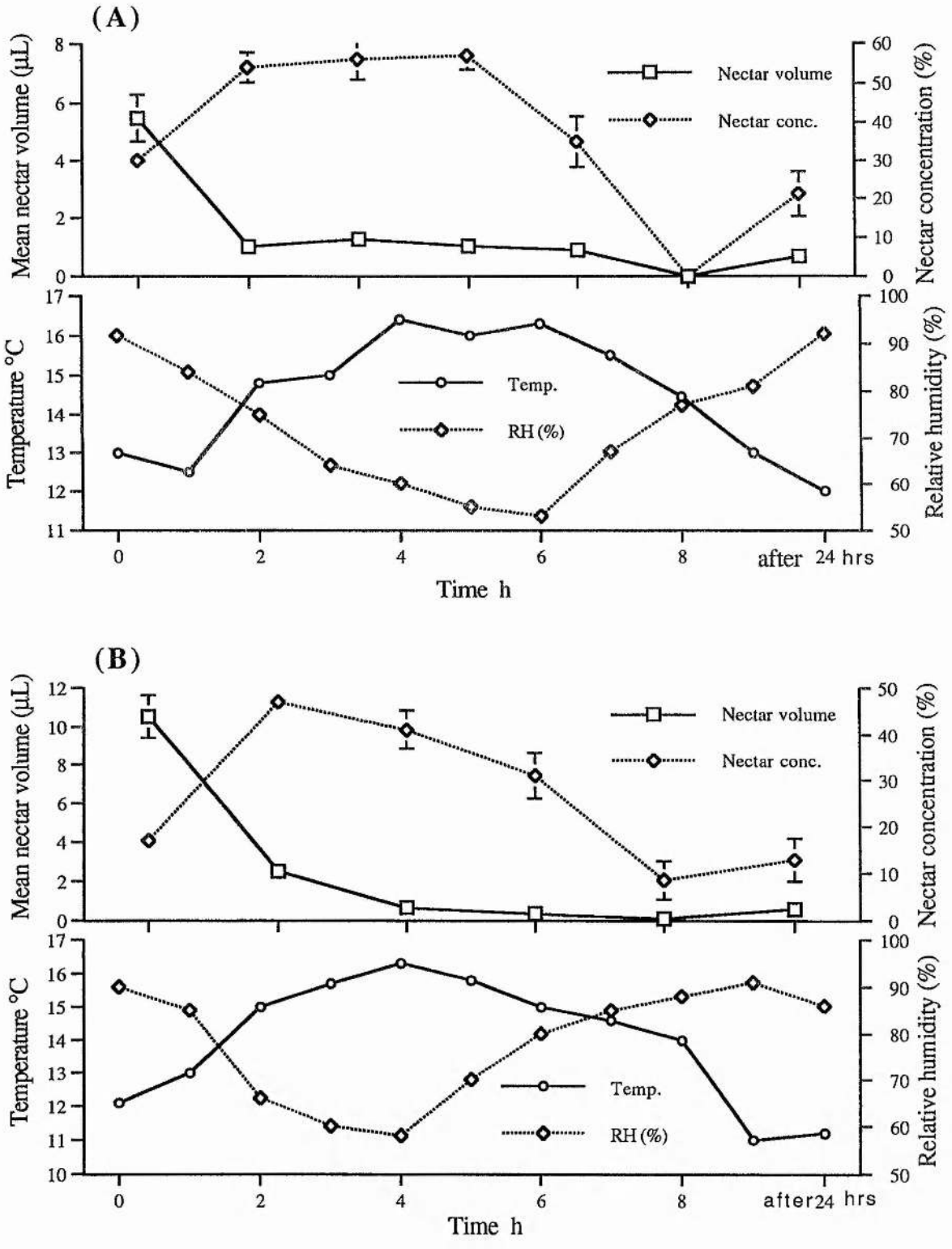


Fig. 4.3a Changes in the volume and concentration of nectar in bagged young flowers of Glen Moy through two different days (29.5.1992 (A) and 4.6.1992 (B)) and after 24 hrs from the first sampling, starting 0800. Showing Mean ± SEM from 20 flowers at each sampling time in relation to the prevailing temperature and relative humidity during sampling experiments.

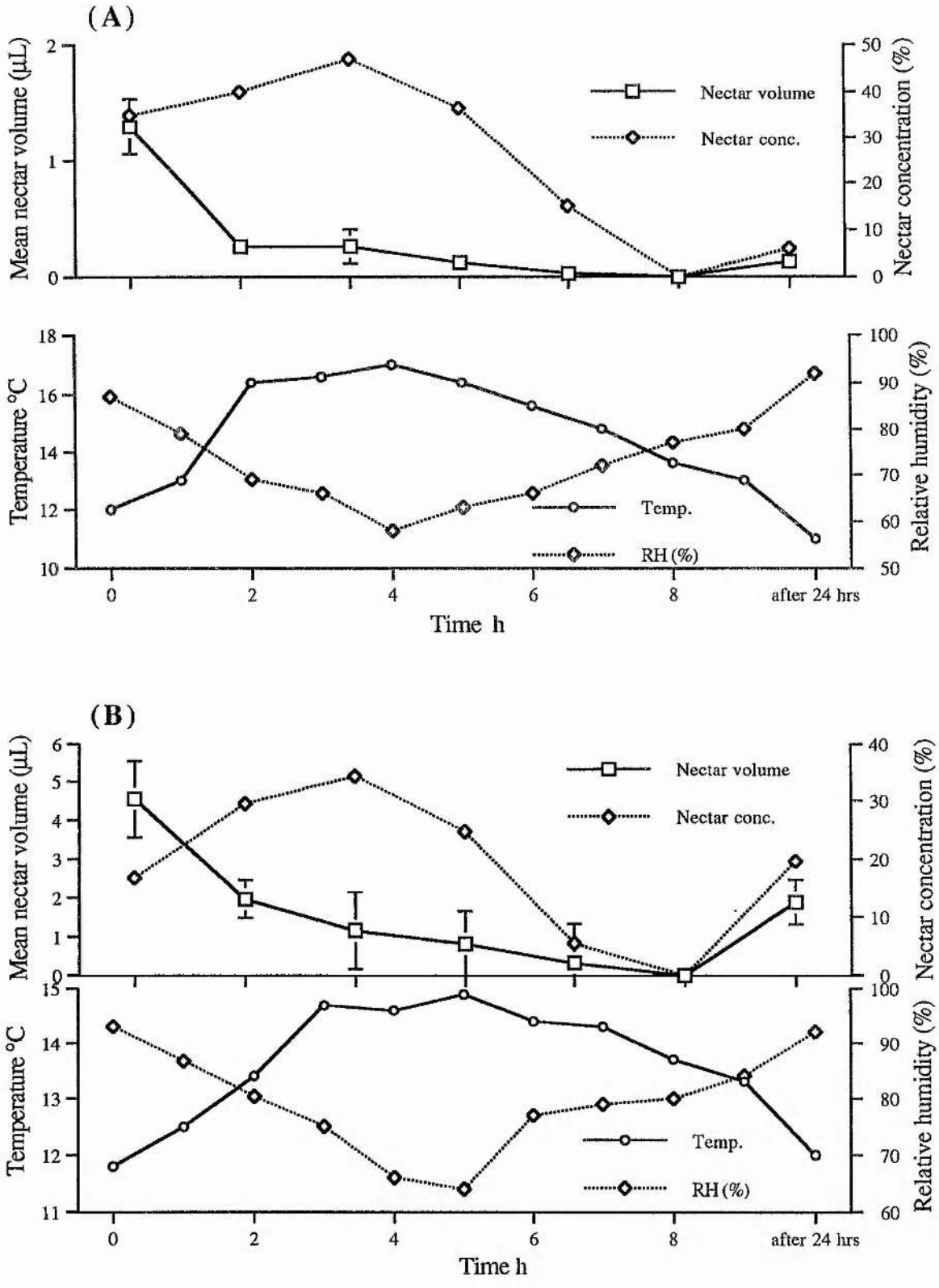


Fig. 4.3b Changes in the volume and concentration of nectar in bagged young flowers of Glen Prosen through two different days (18.6.1992 (A) and 25.6.1992 (B)) and after 24 hrs from the first sampling, starting 0800. Showing Mean ± SEM from 12 flowers at each sampling time in relation to the prevailing temperature and relative humidity during sampling experiments.

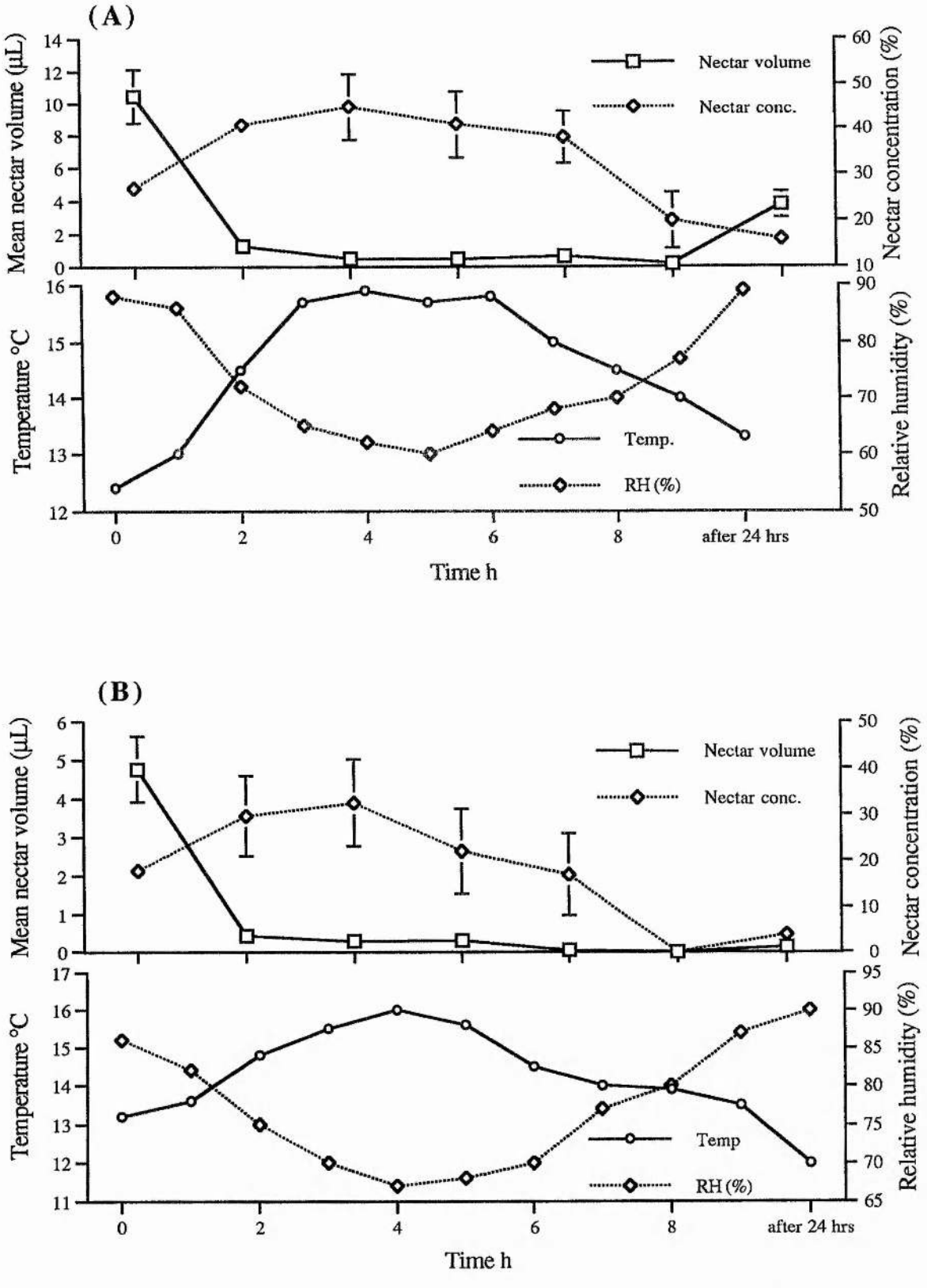


Fig. 4.3c Changes in the volume and concentration of nectar in bagged young flowers of wild raspberry through two different days (15.6.1992 (A) and 22.6.1992 (B)) and after 24 hrs from the first sampling, starting 0800. Showing Mean ± SEM from 16 flowers at each sampling time in relation to the prevailing temperature and relative humidity during sampling experiments.

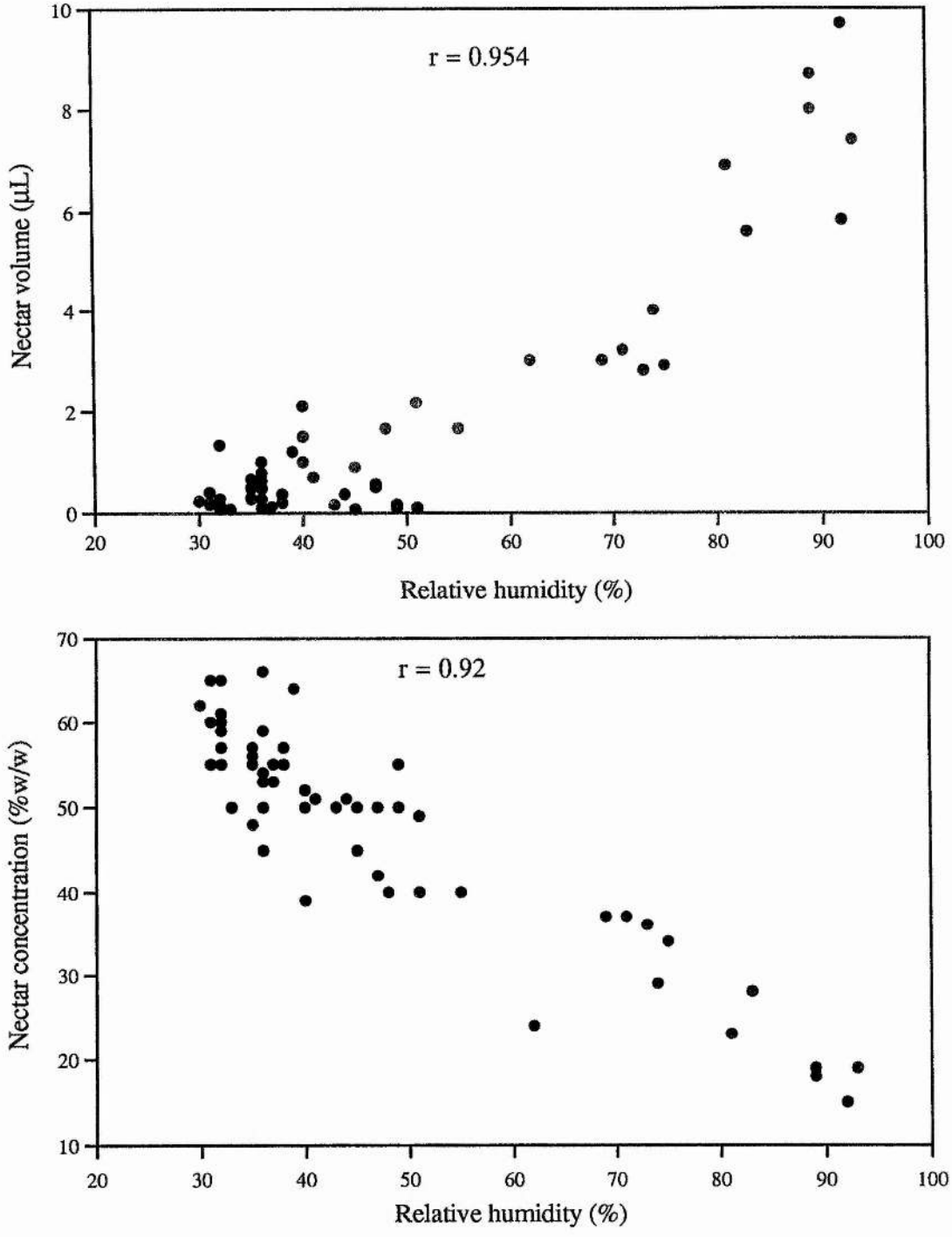


Fig. 4.4a The correlation between the relative humidity and nectar volume and nectar concentration secreted by Glen Moy flowers2 (n = 60).

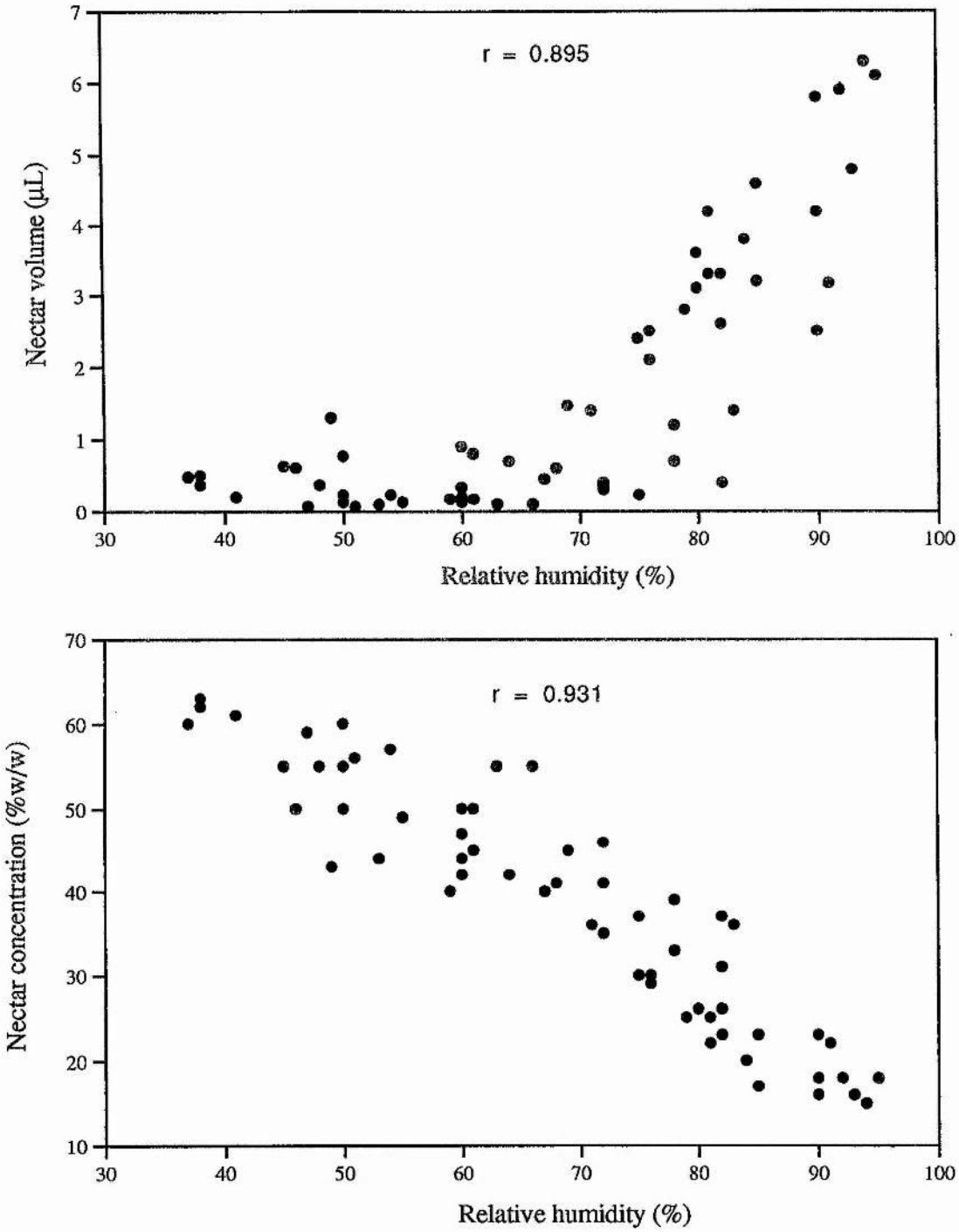


Fig. 4.4b The correlation between the relative humidity and nectar volume and nectar concentration secreted by Glen Prosen flowers (n = 60).

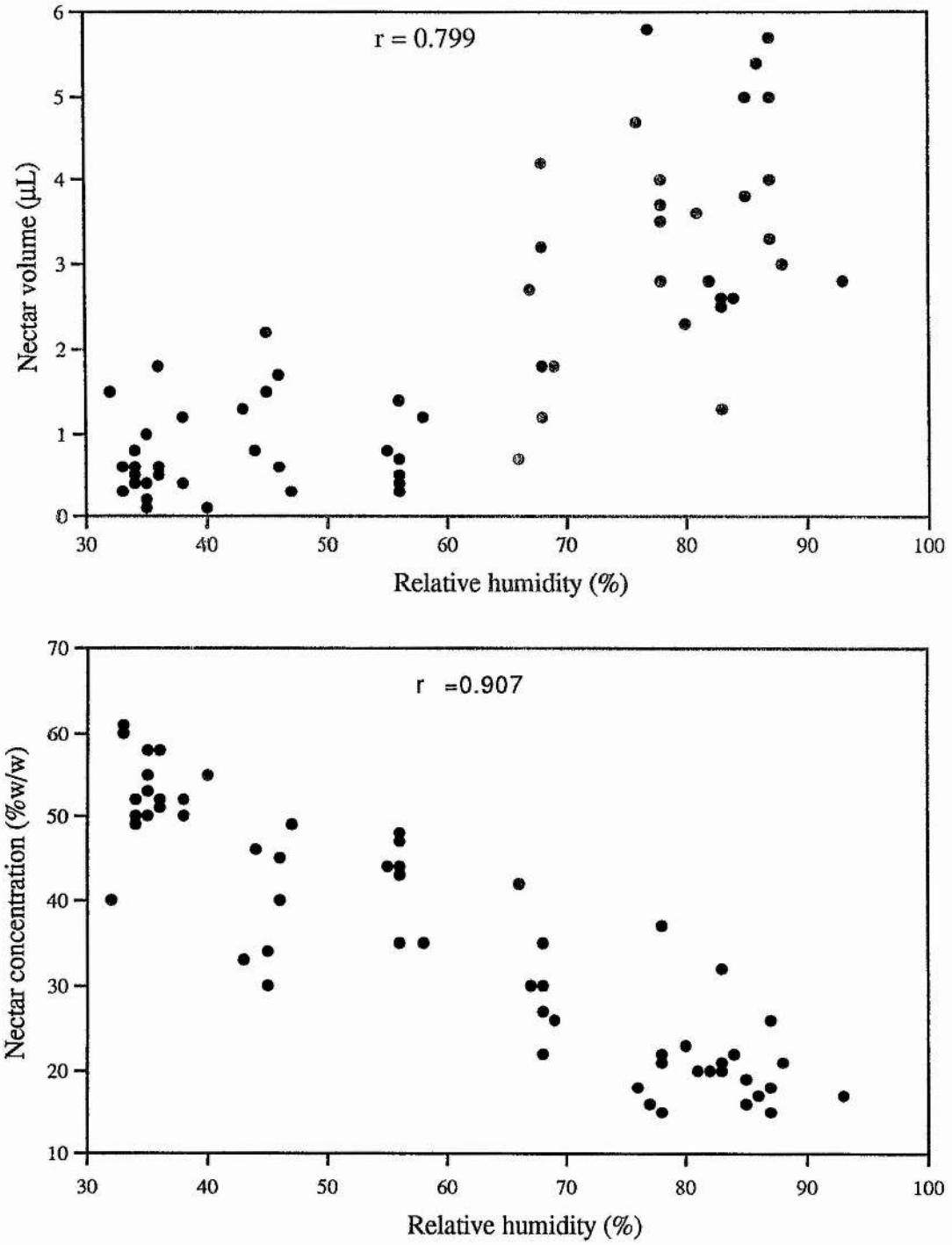


Fig. 4.4c The correlation between the relative humidity and nectar volume and nectar concentration, secreted by wild raspberry flowers. (n = 60).

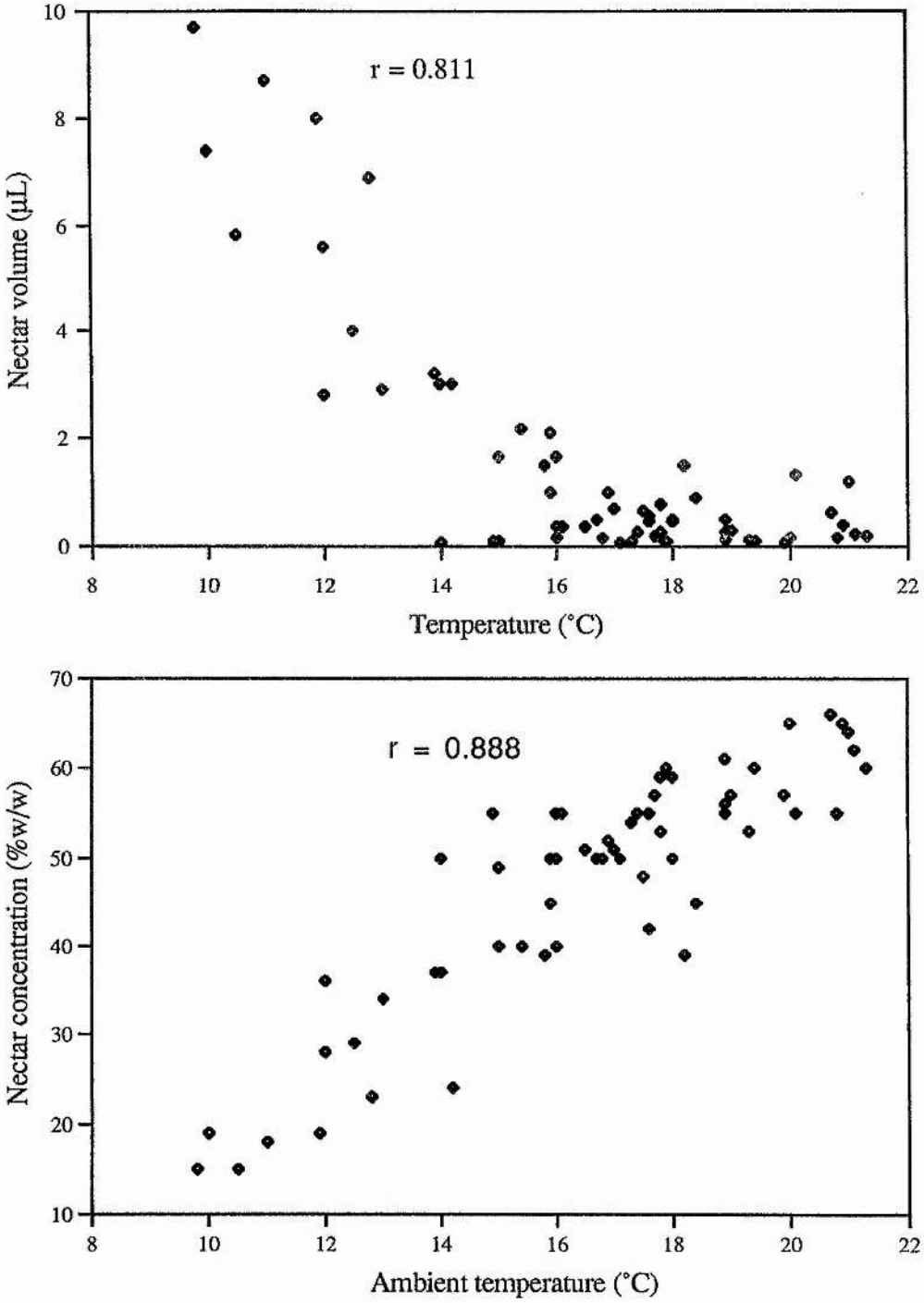


Fig. 4.5a The correlation between ambient temperature and nectar volume and nectar concentration secreted by Glen Moy flowers (n = 60).

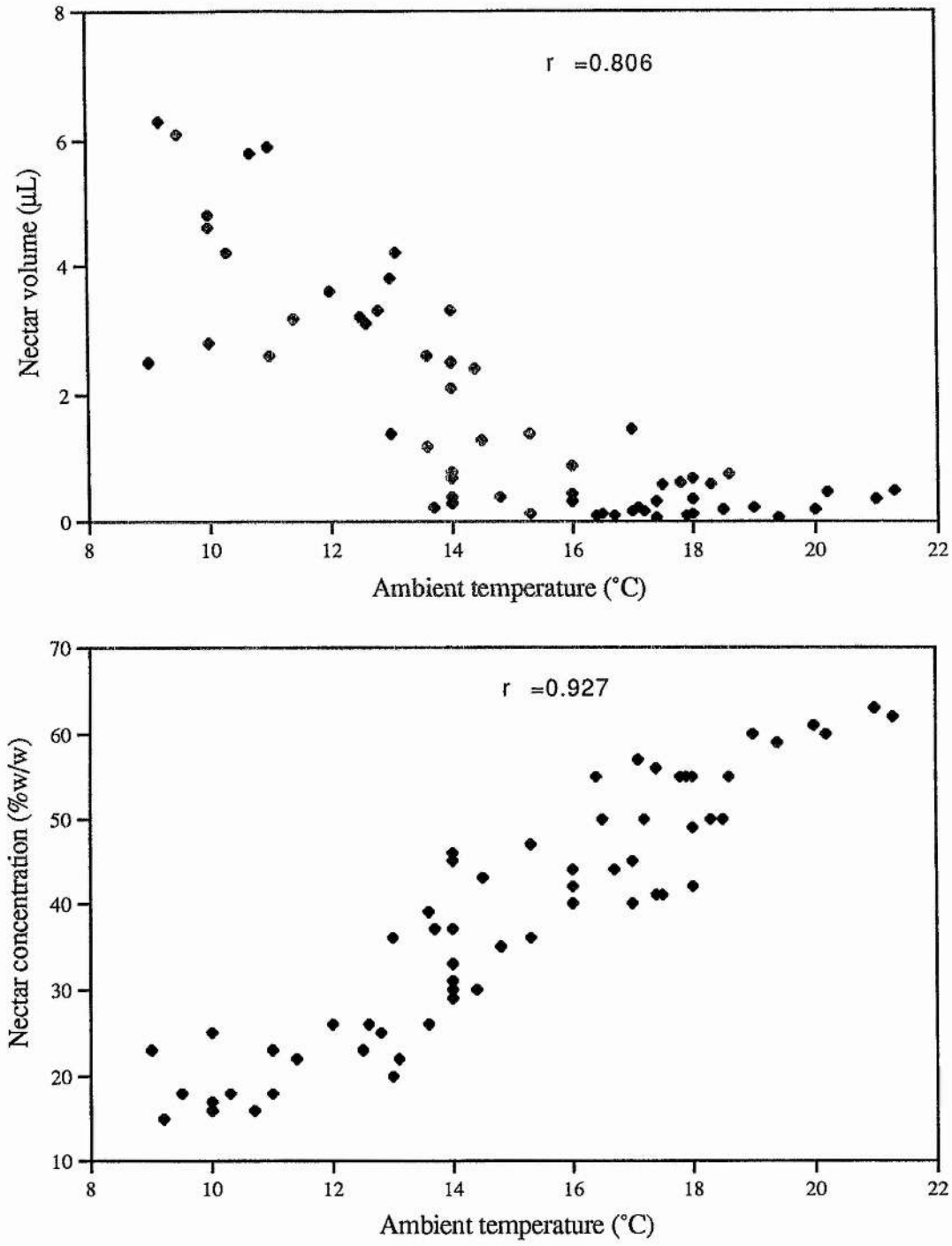


Fig. 4.5b The correlation between the ambient temperature and nectar volume and nectar concentration secreted by Glen Prosen flowers. (n = 60).

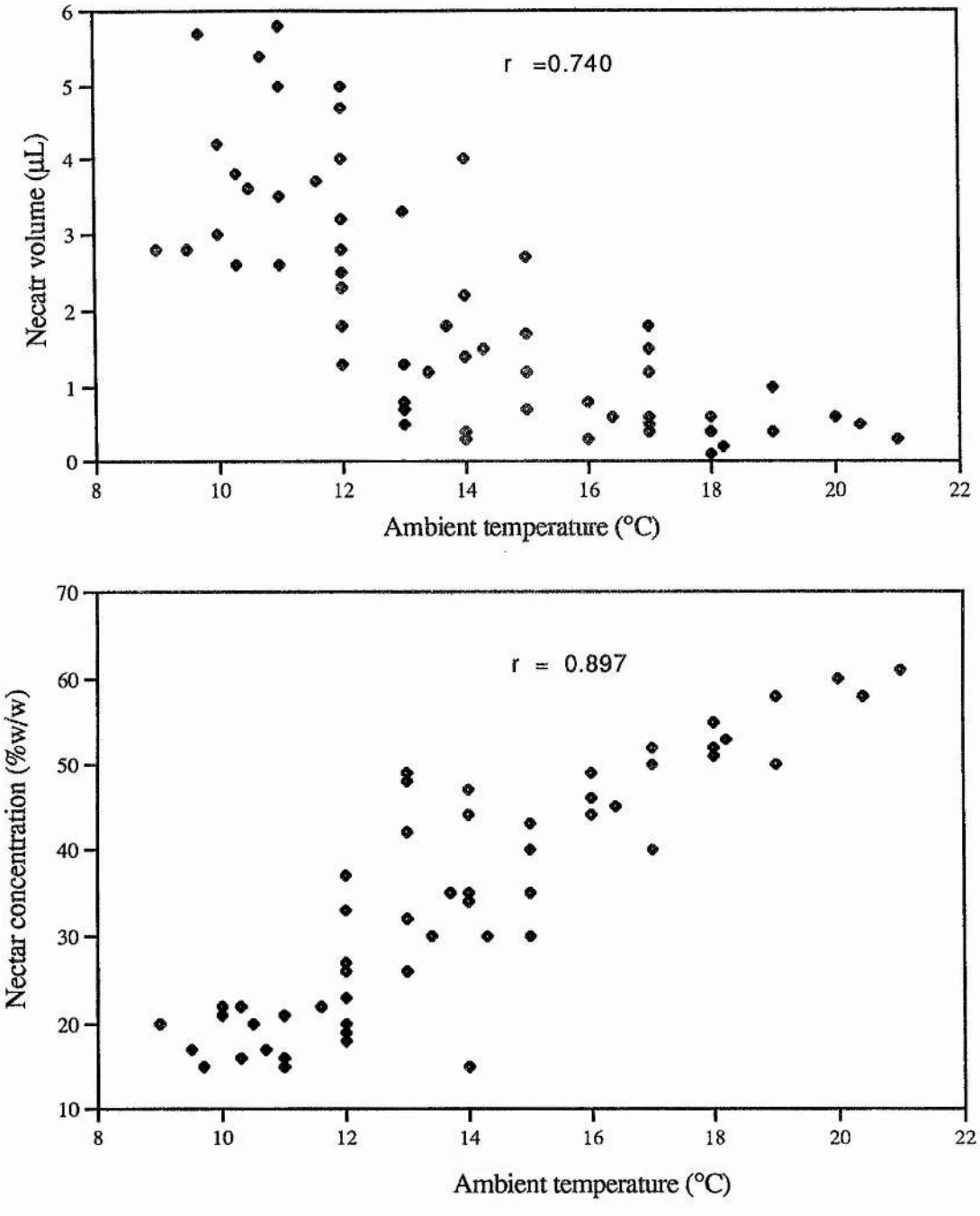


Fig. 4.5c The correlations between the ambient temperature and nectar volume and nectar concentration, secreted by wild raspberry flowers (n = 60). Calculated regression lines are shown.

Chapter 5

Visitor abundance

5.1. Introduction

5.2. Insect diversity.

5.3. Seasonal patterns of insect activity.

5.3.1. Seasonal visitor abundance.

5.3.2. Patterns of *Bombus* species throughout the flowering seasons.

5.4. Daily abundance.

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5.4.2. Diurnal foraging pattern of *Bombus* species.

5.4.3. Diurnal foraging pattern of insect visitors in relation to microclimate conditions and nectar production.

5.5. Some factors affecting the activity of bees on raspberry flowers.

5.5.1. The effects of flower number on bee activities.

5.5.2. The effects of nectar production on bee activities.

5.5.3. The effects of temperature and relative humidity on insect activity.

5.6. Discussion

5.1. Introduction

Foraging patterns of animals may vary in response to the conspicuousness, abundance and spatial patterns of their resources as well as the abundance and spatial patterns of other consumers (Morse 1980). Many factors could affect the foraging pattern of the raspberry visitors. Nectar in the flower acts as an attractant to insects, and in effect assists in bringing about cross-pollination. More insects are attracted to the flowers by the nectar they produce than by pollen (Percival 1950). Thus the presence of nectar in flowers is likely to lead to an increase of visitors (Mosquine 1971; Cruden 1976; Heinrich 1976a,b,c; Morse 1980). Brink and de Wet (1980) working on *Aconitum columbianum* showed clearly that differences between populations in quantity of nectar produced are correlated with differences in the pollinators visiting the plant.

A plant that presents a greater nectar reward is more attractive for its pollinators. An individual plant's probability of visitation will be affected by two factors: the total level of pollinator activity in the surrounding population and the degree to which pollinators select that plant to visit (Schmitt 1983). The number of flower visits may also affect its success in setting outcrossed seed. Optimal foraging theory predicts that a pollinator forages in a way that maximises its net rate of energy intake while foraging (e.g. Pyke *et al* 1977). According to this, the ratio of pollinators to flowers in a patch should be affected by energetic constraints (Pleasants 1981), and a higher level of pollinator visitation can be expected in a flower patch yielding higher rates of energy gain, so that pollinator visitation should be greater in patches of higher flower densities. Moreover, pollinators may be differentially attracted to plants with greater numbers of flowers (Willson & Rathcke 1974; Schaffer *et al* 1979).

Pollinator activity may also be affected by microclimatic interactions with physiological constraints, particularly for insect visitors (Willmer 1982). For example Willmer (1986) provided evidence for microclimatic effects on fluctuations in body fluid concentration of bees. A study by Heinrich (1976b) showed bumble bees foraging in cooler conditions than was possible for smaller solitary bees, and similar effects can be seen in the recorded visits to Convolvulaceae described by Schlising (1970). Teras (1976) and Benedek & Prenner (1972) provide evidence that flight activity of insects increases with rising temperature, and that the flower visiting speed of honey bees increases with rise in temperature.

In this chapter I will address many questions.

1 - Do the red raspberry flowers attract diverse insect visitors ?

2 - Do the individual species of visitors show the same relative abundance when they visit different raspberry cultivars?

3 - How can the microclimate conditions (temperature and humidity) affect the activity of insect visitors on raspberry flowers throughout the flowering season?

4 - What is the effect of raspberry flower number on relative abundance of insect visitation?

5 - What is responsible for the attractiveness to insects of raspberry flowers, nectar concentration or nectar volume?

Also I investigated the seasonal, daily and hourly changes in insect visits to the raspberry cultivars in relation to changes in nectar production and microclimatic conditions.

5.2. Insect diversity

The survey of insect visitors to flowers of cultivated and wild raspberry at SCRI and Cameron reservoir revealed that the unsprayed raspberry attracted a wide range of insect visitors. Extensive collecting, representing 25 species during the season 1992, 1993 and 1994, provided a sound data base for an analysis of the structure of the plant pollinator community (Table 5.1). The major flower visitors and possible pollinators were bees (families Apidae, Andrenidae and Halictidae) and flies (family Syrphidae). Beetle visitors also fed on nectar, the most frequently observed being *Byturus tomentosus*, the raspberry beetle, a destructive pest whose eggs are laid in the flowers (Willmer, Bataw and Hughes 1994); and the ladybird *Coccinella 7- punctata*.

There were relatively few differences between the insect visitors to the three cultivars. Wild raspberry was attractive to more different species of hover flies than Glen Prosen and Glen Moy flowers, and that could be due to the flowering diversity around the wild raspberry area. *Psithyrus vestalis* was attracted to the wild flowers only. Most of the hover fly species' abundance was very low and their activities were not continuous, some of the species being observed on only a few days throughout the whole season. The only consistently abundant species of Syrphidae was *Syrphus ribesii*. Despite many *Halictus rubicundus* nests very close to the cultivated raspberry area (about 5 meter), the activity of the *Halictus* on raspberry was very rare; this needs further investigations but the nectar concentration and/or composition might be one of the reasons.

5.3. Seasonal patterns of insect activity.

The abundance of pollinator groups was estimated as the number of arrivals to raspberry cultivar flowers by individual insects of that group during

the observation periods (see below). Thus abundance is not equivalent to population size but is a measure of activity at raspberry flowers.

5.3.1. Seasonal visitor abundance.

The abundance of insect visitors was studied throughout the seasons 1992, 1993 and 1994 for the three raspberry cultivars. The seasonal abundance of insect visitors to Glen Moy flowers during these seasons is shown in fig 5.1: all the three seasons show low abundance of insect visitors in the early and late parts of the flowering periods. In the season 1992 the insects peaked in the middle of the season. On 11,13 and 16 June, *Bombus* spp. and *Apis mellifera* were most abundant, while *Andrena* spp showed more activity in the early and middle season and its abundance declined at the end of season. In 1993 the *Bombus* spp and *Apis mellifera* peaked around 10 June with about 80 and 43 bees respectively recorded during 30 min per 1 meter. In 1994 *Bombus* spp and *Apis mellifera* peaked on 16 June while *Andrena* spp peaked on 11 June. All the recording days show that the numbers of *Bombus* species were greater than for any other insect visitors.

The visitors to Glen Prosen flowers (fig 5.2) show a difference between the three different seasons. In the season 1992 the number of visitors was higher than the two other seasons, and all the three seasons show high abundance of the insect visitors in the early season, sharply increasing then declining gradually. All the recording days shows that the numbers of *Bombus* species were the highest among the other insect visitors followed by *Apis mellifera* then *Andrena* species and hover flies.

Fig 5.3 shows the activity of insect visitors to wild raspberry flowers throughout the seasons 1992, 1993 and 1994. The data again illustrate that the number of insect visitors during the season 1992 was higher than for the other

seasons. In 1992, *Bombus* showed highest activity on 15, 18 and 22 June, while *Apis mellifera* showed highest activity on 15 and 18 June. In 1993 *Bombus* and *Apis* showed highest activity on 11 and 15 June, while in 1994 *Bombus* showed highest activity on 15 and 18 June while *Apis* showed high abundance on all the recording days between 12 and 23 June. In 1992 and 1993 *Andrena* and hover flies showed more activity in the middle of the season, while in 1994 they showed highest activity at the beginning of the season. Also for wild raspberry the major visitors were *Bombus* species followed by *Apis mellifera*, *Andrena* species and hover flies.

A comparison between the relative abundance of insect visitors to the three raspberry cultivars is given in fig 5.4. Here the mean numbers of visitors per 30mins for each day are summed for all recording days in a season. Although numbers of recording days vary slightly, this figurs illustrates that the total mean number of visitors in the flowering season 1992 was more than the number of insect visitors attracted to the raspberry flowers in the other two seasons. *Bombus* species were predominant in all the three seasons. For example the percentage of *Bombus* species in the total insect visitors to Glen Moy during 1992 was 62.5%, followed by *Apis mellifera* 26.5%, *Andrena* spp 8.0% and hover flies 3.0%. In 1993 the *Bombus* spp also showed more abundance (60.1%), with *Apis mellifera* 24.5%, *Andrena* spp 9.8% and hover flies 5.6%; and in 1994 the percentages of *Bombus* spp were 55.1%, *Apis mellifera* 33.1%, 6.8% for *Andrena* spp and 5.0% for hover flies.

The percentages of *Bombus* as visitors to the three raspberry cultivars fluctuated from one cultivar to another; Glen Moy flower showed highest overall attractiveness to *Bombus* species (59.2%) followed by wild raspberry flowers (53.6%), while Glen Prosen showed the lowest value (50.3%).

Apis mellifera made up an almost constant percentages of the total visitors on the three cultivars; on wild raspberry they were 29.8% of the total insect visitors, 29.0% on Glen Prosen and 28.0% on Glen Moy. *Andrena* species showed highest selective abundance on Glen Prosen (11.7%), followed by wild raspberry (8.6%) and Glen Moy (8.2%)

The data also illustrate that the abundance of hover flies was highest on Glen Prosen (9.0%) and wild raspberry (8.0%), but rather low for Glen Moy (4.6%). That could be due to a mismatch between the earlier flowering of Glen Moy and the relative lateness of hover flies' seasonal activity.

The activities of insect visitor' on raspberry flowers show clear declines from year to year. This might be due to many factors e.g. weather conditions, or the ageing of the raspberry plant which showed declines in flower production (see chapter 3). There also may be some factors relating to the number of bumble bee nests and their abundance around the studied sites, and to the activities of beekeepers in the case of honey bees.

5.3.2. Patterns of *Bombus* species throughout the flowering seasons.

Fig 5.5 (a) shows the numbers of individual species of *Bombus* foraging on Glen Moy flowers throughout the different flowering seasons. *Bombus lucorum*, *lapidarius* and *terrestris* were predominant over the other *Bombus* species; 32.5% of the total *Bombus* visitors were *Bombus lucorum*, followed by *Bombus lapidarius* 30.6% and *Bombus terrestris* 26.3%, with *Bombus pratorum* at 7.4% and *Bombus pascuorum* at 3.6%. The activities of *Bombus* species showed variable patterns throughout the different seasons, *Bombus lapidarius* showing highest activity in the season 1994, while *lucorum* showed highest

activity in 1992 and 1993. *Bombus pascuorum* was the least abundant bumble bee on Glen Moy flowers throughout the different seasons.

On Glen Prosen fig 5.5 (b) illustrates that *Bombus lucourum, lapidarius* and *terrestris* again were the dominant bumble bee species; about 33.5% of the total *Bombus* was *lucorum* followed by *Bombus lapidarius* 30.2%, and *B. terrestris* 25.6%, *B. pratorum* 6.4% and *B. pascuorum* 4.3%.

Fig 5.5 (c) illustrates that the pattern of bumble bees abundance on wild raspberry flowers was the same as on cultivated flowers. *Bombus lucorum* was predominant at about 33.7% of all visits followed by *lapidarius* 30.3%, *terrestris* 25.8%, *pratorum* 3.9% and *pascuorum* 2.8%.

5.4. Daily abundance.

5.4.1. Diurnal foraging pattern of insect visitors on different days.

Complete records of visitors were made in 1992 on two days for each raspberry cultivar. The data show the diurnal foraging patterns of insect visitors, in terms of mean number of insect arrivals to the patch (1 meter) during 15 min.

a. Glen Moy

On 29.5.92, bees showed high activity during the daylight hours (fig 5.6), especially in the morning and middle of the day. The activity reached its peak between 1000h and 1200h, then started to decline. *Bombus* was more active in the morning and with some activity observed in the evening. *Apis mellifera* was more abundant around midday. but its activity was quite low in the evening, probably due to the drop in temperature. The activities of *Andrena* spp and of hover flies were very low in the morning and evening, with peaks in the warmest hours of the early afternoon.

The overall activity of the insect visitors on 4.6.1992 was again morning and midday with some activity in the evening (fig 5.6). The activity of insect visitors was higher than on 29.5.1992, perhaps because of slightly warmer weather, but all the insect visitors showed the same foraging patterns, the highest activity of *Bombus* species being concentrated in morning and evening while the activities of *Apis*, *Andrena* and hover flies were in the warmer periods of the day.

b. Glen Prosen

Fig 5.7 shows that the overall activity of the insect visitors on 18.6.92 was highest in the morning, with a secondary smaller peak in the evening. The activity of *Apis mellifera* was low until 1000h when it increased gradually with increased temperature to peak at 1400h and then decline. The bumble bees started activity earlier, peaking at 1000h and then declining till 1400h; they increased again till 1800h then declined. The activities of hover flies and *Andrena* were concentrated around mid day.

On 25.6.92 (fig 5.7) the activities of the insect visitors showed similar patterns, though *Apis*, *Andrena* and syrphids were more abundant in the morning, when temperatures were higher than on 18.6.92. But again *Bombus* dominated the early morning and evening, with the other visitors concentrated in the middle of the day.

c. Wild raspberry

The activity of insect visitors on 15.6.92 started before 0800h, and was highest between 1000h and 1200h (fig 5.8). *Apis mellifera* and *Bombus* were more abundant than the other visitors. *Bombus* spp shows more activity in the morning, while *Apis mellifera* peaked around 1200h when the temperature reached 18°C. Hover flies and *Andrena* spp appeared throughout the period of

observation with low numbers, and they also peaked around mid day. Very similar patterns were observed on 22.6.92 (fig 5.8).

For all cultivars, the insects activity throughout the different days showed changes from hour to hour. Maximum insect activity, for most species, occurred between 0800h and 1800h, and for many of them activity was not observed at all earlier than 0800h, especially *Apis mellifera* and *Andrena* spp. This is probably due to low temperatures (and perhaps high relative humidity) outside these hours.

In general *Bombus* species collectively had a markedly longer active period than *Apis* on most days of observation, with *Apis* much more frequent in the warmer parts of the day (1200-1800h). In the next section I will analyse in detail the activities and the abundance of individual *Bombus* species measured on the same observation days.

5.4.2. Diurnal foraging patterns of *Bombus* species.

The specific bumble bee activities were measured to assess which were the most active individual species of *Bombus* on Glen Moy, Glen Prosen and wild raspberry flowers on a two different days.

On 29.5.92 for Glen Moy fig 5.9 shows that *Bombus lucorum*, *lapidarius* and *terrestris* were more active than the other *Bombus* species. All species showed the same basic pattern of bimodal activity, with large morning peaks and smaller evening peaks.

The activities of individual *Bombus* spp. on Glen Moy were studied 7 days later on 4.6.92 (fig 5.9), and the data again illustrated that most of the species show activity in the beginning of the day and up to midday, the activity after noon showing a decline, with a further small peak in the evening. The

dominant species were again *lapidarius*, *lucorum* and *terrestris* while *pratorum* and *pascuorum* showed less abundance.

Bombus foraging on Glen Prosen on 18.6.92 and 25.6.92 (fig 5.10) showed higher abundance in the morning, though the early peaks were sometimes less obvious

Individual *Bombus* visiting wild raspberry flowers (fig 5.11) on 15.6.92 and 22.6.92 showed the same basic patterns as when they visited cultivated raspberry flowers). However, the bimodal character of visitors' abundance was often reduced on wild plants, with a gradual decline from the morning peak numbers and little evidence of an evening peak for any species.

5.4.3. Diurnal pattern of insect visitors in relation to microclimate conditions and nectar production.

Different factors could affect the activity of insect visitors in the field. Floral nectar serves two functions, attracting pollinators and affecting the duration of their visits, which indirectly governs pollen receipt and donation (Thomson *et al* 1986). Volumes and concentrations of nectar in individual flowers are usually quite good predictors of nectar volumes in other flowers on the same plant. This relationship provides an indirect method for examining the patterns of nectar collection by visitors as a function of a flower's reward. This experiment was designed to investigate the effects of the pattern of nectar availability and microclimate conditions on insect visitors in the field throughout one day for each raspberry cultivar. The nectar was taken from raspberry flowers in different flower stages. I sampled the nectar in the field without bagging the flowers, to estimate actual nectar available for pollinators. I have included only the insects collecting nectar in this part of the study.

The volume and sugar content of nectar in the flowers of Glen Moy, Glen Prosen and wild raspberry followed similar patterns (fig 5.12, fig 5.13 and fig 5.14). Both volume and sugar content were highest at 0800h and both fell to their lowest level around 1200 - 1400h on Glen Moy and Glen Prosen, and about 1600 h on wild raspberry. Individual flowers of all the three cultivars varied considerably in the amount of nectar they contained, especially in the early morning, at 0800 h, when secretion was at its highest; flowers of Glen Moy on the same branch could contain between 1.6 - 9.7 µl., Glen Prosen 1.1 - 3.3 µl. and wild raspberry 1.3 - 6.7 µl.

Nectar concentration was always rising around mid day due to the drying effect of low relative humidity in all the three cultivars. Concentration in Glen Prosen peaked earlier in the day (1000 - 1400 h) than did Glen Moy (1200 - 1600 h) and wild raspberry flowers (1200 - 1400 h).

The records from raspberry flowers show that nectar was secreted in the morning and evening at a nectar concentration of about 40 - 49% sugar in Glen Moy flowers; 26 - 35% sugar in Glen Prosen; and about 22 - 32% sugar in wild raspberry. Secretion generally coincided with low temperature and high relative humidity in these periods. The overall range of sugar concentration of nectar in Glen Moy, which was between 40 - 57% sugar, was much higher than Glen Prosen (26 - 53% sugar), and wild raspberry (22 - 51% sugar), but since the measurements were carried out on different days the difference may not be of significance.

Most insect visitors to the three raspberry flowers showed the same trend in their activity, in that they tended to visit around the middle of the day. Their numbers thus increased gradually with increased temperature and nectar concentration. The exception was again *Bombus* species which were less

common around midday and visited more in the early morning and late evening when the maximum nectar volume per flower was available.

The question therefore arises whether nectar volume or nectar concentration is responsible for insect visitation patterns on raspberry flowers? The answer will be discussed below.

5.5. Some factors affecting the activity of bees on raspberry flowers.

Many factors could be affecting the activity of insects, some relating to the plant, some to microclimate and some to the insects themselves. In my study I concentrate on effects of flower number on the frequency of insect visits, the effects of floral reward quality, and the effects of ambient temperature and relative humidity.

5.5.1. The effects of flower numbers on bees activities.

In this section, I investigated pollinator responses to effective patch size (i.e., the relationship between mean number of flowers/1m and the mean number of visitors to that 1m patch over 30 min.). Fig 5.15 shows using a linear regression that the number of flowers was a good predictor of most visitors' abundance on raspberry flowers. The mean number of Glen Moy flowers was highly significantly correlated with the mean number of *Bombus spp.* ($r = 0.972$, $P < 0.05$), *Apis mellifera* ($r = 0.955$, $P < 0.05$) and also with *Andrena* spp. ($r = 0.960$, $P < 0.05$).

Similarly high correlation coefficients were obtained for Glen Prosen flowers (fig 5.16), and for wild raspberry flowers (fig 5.17) (statistics are shown on the figures)

Thus, in general, at the beginning of the flowering season when the number of flowers were low, the numbers of visitors attracted to the flowers were very low and gradually when the number of flowers were increasing the number of visitors obviously also increased.

Plants with high mean number of flowers per meter are presumably more attractive to pollinators since they offer more rewards per unit area than plants of low flower density.

5.5.2. The effects of nectar production on bee activities.

The effects of nectar volume (µl.) and nectar concentration (%) on the bees activities on the three raspberry cultivars were determined by sampling the nectar three times a day (morning, mid day and afternoon) throughout different days during the season 1993 in relation to the relative abundance of insect visitors. The data represent the insect visitors that seek nectar only, and I ignored the insect visitors which were searching for pollen.

Table 5.2. shows the correlations between the number of bees visiting raspberry flowers and their nectar volume and concentration. Bees were responding to both nectar volume and nectar concentration in all the three cultivars. The data demonstrate that numbers of honey bees, *Andrena* species and hover flies were related positively to the nectar concentration, and foraged at the times when the nectar concentration was high, avoiding foraging in the periods when the nectar was diluted. *Apis mellifera*, *Andrena* species and hover flies showed negative relations with the nectar volume.

However *Bombus* species showed negative responses to concentration and they foraged when the nectar concentration was low. *Bombus* species instead showed highly significant positive correlations with nectar volume on all the three raspberry cultivars.

Thus most kinds of insect visitors pattern their activities in relation to nectar concentration (which is highest around midday to early afternoon) but *Bombus* species are more concerned to acquire high nectar volume, which is inversely correlated with concentration and peaks in the morning and evening.

5.5.3. The effects of temperature and relative humidity on insect activity.

Willmer (1982) stated that temperature of the environment and its water content (relative humidity) were the major climatic parameters, and that these factors were continuously modified by the other two major climatic variables, wind and solar radiation. Also she stated that temperature and relative humidity can be directly correlated with insect activity. In order to study the effects of weather conditions on insect activity on raspberry flowers, experiments were carried out during the 1993 flowering season. The records of air temperature and relative humidity in relation to insect activity were taken three times through recording days (in the morning 0800h - 1000h, mid day 1200h - 1400h and afternoon 1600h - 1800h) this covered the activity periods of insect visitors. In this experiment I am not interested in determining the threshold at which the bees can be active, but my interest concentrated on the effects of prevailing weather conditions. The correlation coefficients were calculated between temperature, humidity and number of bees foraging on Glen Moy, Glen Prosen and wild raspberry flowers, by using all measurements collected during the recording days from SCRI and Cameron reservoir sites.

Table 5.3 shows that the bees responsed variously to the temperature and humidity. *Apis mellifera* and *Andrena* spp were strongly affected by temperature and humidity on all three cultivars, i.e. their foraging depended on temperature and relative humidity. *Bombus* spp showed much less ''concern'' with weather patterns, and their abundance was negatively correlated with temperature.

5.6. Discussion.

The three raspberry cultivars all attracted diverse insect visitors, the most abundant being bumble bees (*Bombus lapidarius*, *B. lucorum*, *B. terrestris*, B. *pratourus* and *B. pascuorum*), *Apis mellifera*, *Andrena* species and hover flies. Bumble bees were responsible for about 60% of all visits, with honey bees, *Andrena* and hover flies making up most of the remaining visits. *Bombus* species were more abundant through the particular observation days and through the different seasons, and they were present at almost all times of observations.

The insects visiting raspberry flowers feed mainly on nectar (chapter 6) and are likely to visit Glen Moy cultivars more frequently as it produces more nectar than Glen Prosen and wild raspberry flowers (chapter 4).

Seasonal patterns of insect visitation follow the abundance of raspberry blossom, peak insect visitors' abundance corresponding to peak flowering of the three cultivars. Bees showed high abundance on the raspberry flowers while Syrphidae showed lower abundance. Bumble bees were predominant among the bees attracted to raspberry flowers. *Bombus* species were variable in their activity from one cultivar to another and from one season to another; *Lucorum* was most abundant in 1992-1993, but *lapidarius* became increasingly common as my studies progressed, reflecting its general recent population grouth in Scotland (Willmer, pers. comn.).

Bumble bees are commonly active all through the daylight hours and may even forage busily before sunrise or after dusk (Sladen 1912). However, before 0700h and after 1900h flower visits are usually scarce (Free and Butler 1959). Between these hours, bumble bees are sometimes said to forage with the same activity throughout the day (Loken 1949), and some studies show that most *Rubus* visit are made shortly after midday (Teras 1976). My investigation agrees with studies which documented that the bumble bees are active all

through daylight on the three raspberry cultivars, peaking early morning and evening.

Apis mellifera and *Andrena* spp were usually only active after 0800. Variation in the number of *Apis mellifera* was directly proportional to temperature and inversely proportional to the relative humidity (c f. Ciurdarescu 1971). *Bombus* species, which have the ability to warm up independently (Heinrich 1976b; 1993; Willmer 1983), showed little dependence on weather and they were more active early and late in the day when it was cooler. Thus they behaved differently from *Apis*, *Andrena* and hover flies.

Because rare flowers might receive few visits (Levin 1972, Silander 1978), an increase in floral density of raspberry flowers can increase visitation rate of the bees. Flower number can affect the quantity of visits received by individual flowers (Sih & Marie-Sylvie 1987), and this would indicate that one of the ways in which a plant can increase its attractiveness to pollinators is to produce a large number of flowers. Pollinators generally track flower density (Thomson 1981, 1982; Stephenson 1982; Gross and Werner 1983; Rathcke 1988), although visitation rates of pollinating bees generally vary in their responses to plant density; therefore, peak visitation does not match peak flowering (Thomson 1981; Stephenson 1982). Some studies have documented that patterns of bee visitation showed no association with seasonal patterns of flower density (Dieringer 1991). Eckhart (1992) and Willson & Bertin (1979) indicated that the arrival rate of visitors increases with flower number. For the three raspberry cultivars flowers there is a highly significant correlation between the number of flowers produced by the plant and the bee activities.

In conclusion, for *Apis*, *Andrena* and hover flies the main factors favouring abundance are high flower density, high temperatures, and high nectar

concentrations. *Bombus*, in contrast, are favoured by high flowers density again but also by cooler temperatures, low concentration and higher nectar volumes.

Table 5.1. Insect diversity visiting cultivated and wild raspberry flowers during the flowering seasons 1992, 1993 and 1994. (+) recorded (-) not recorded

a-

Hymenoptera	Glen Moy	Glen Prosen	wild
B. lucorum	+	+	+
B. lapidarius	+	+	+
B. terrestris	+	+	+
B. pratorum	+	+	+
B. pascuorum	+	+	+
Psithyrus vestalis	-	-	+
Apis mellifera	+	+	+
Andrena nigriceps	+	+	+
Andrena albicans	+	+	+
Halictus rubicundus	+	+	+

b-

Diptera	**Glen Moy**	**Glen Prosen**	**wild**
Syrphus ribesii	+	+	+
Syrphus vitripennis	+	+	+
Metasyrphus corollae	-	-	+
Metasyrphus luniger	-	+	-
Episyrphus balteatus	-	+	-
Platycheirus manicatus	+	-	-
Parasyrphus punctulatus	-	-	+
Leucozona spp.	-	-	+
Eristalis pertinax	-	-	+
Volucella pellucens	-	-	+
Volucella bombylans	-	-	+
Cheilosia illustrata	-	-	+
Syritta pipiens	-	+	+

c-

Coleoptera	**Glen Moy**	**Glen Prosen**	**wild**
Coccinella 7-punctata	+	+	+
Byturus tomentosus	+	+	+

Table 5.2. A comparison of the correlations of insect numbers with nectar volume and nectar concentration on raspberry flower for all records from all 3 years. A comparison with correlation of nectar volume versus nectar concentration is included. All values are statistically significant ($P<0.05$).

A. Glen Moy

	Nectar concentration (w/w)	Nectar (µl.)
Bombus spp	- 0.52	0.79
Apis mellifera	0.77	- 0.65
Andrena spp	0.72	- 0.60
Hover flies	0.82	- 0.75

Nectar volume versus nectar concentration $r = - 0.78$

B. Glen Prosen

Bombus spp	- 0.84	0.95
Apis mellifera	0.76	- 0.70
Andrena spp	0.77	- 0.65
Hover flies	0.74	- 0.64

Nectar volume versus nectar concentration $r = - 0.83$

C. wild raspberry

Bombus spp	- 0.59	0.93
Apis mellifera	0.90	- 0.55
Andrena spp	0.79	- 0.52
Hover flies	0.75	- 0.50

Nectar volume versus nectar concentration $r = - 0.61$

Table 5.3. The correlation between insect numbers per 10 min and microclimatic conditions for the visitors to the three raspberry flower cultivars. All values are statistically significant ($P<0.05$).

	Temperature (°C)	Relative humidity (%)
Glen Moy		
Bombus species	- 0.42	0.50
Apis mellifera	0.97	- 0.58
Andrena species	0.95	- 0.63
All visitors	0.75	- 0.73
Glen Prosen		
Bombus species	- 0.48	0.59
Apis mellifera	0.93	- 0.53
Andrena species	0.90	- 0.54
All visitors	0.74	- 0.71
wild raspberry		
Bombus species	- 0.46	0.66
Apis mellifera	0.89	- 0.51
Andrena species	0.80	- 0.50
All visitors	0.77	- 0.71

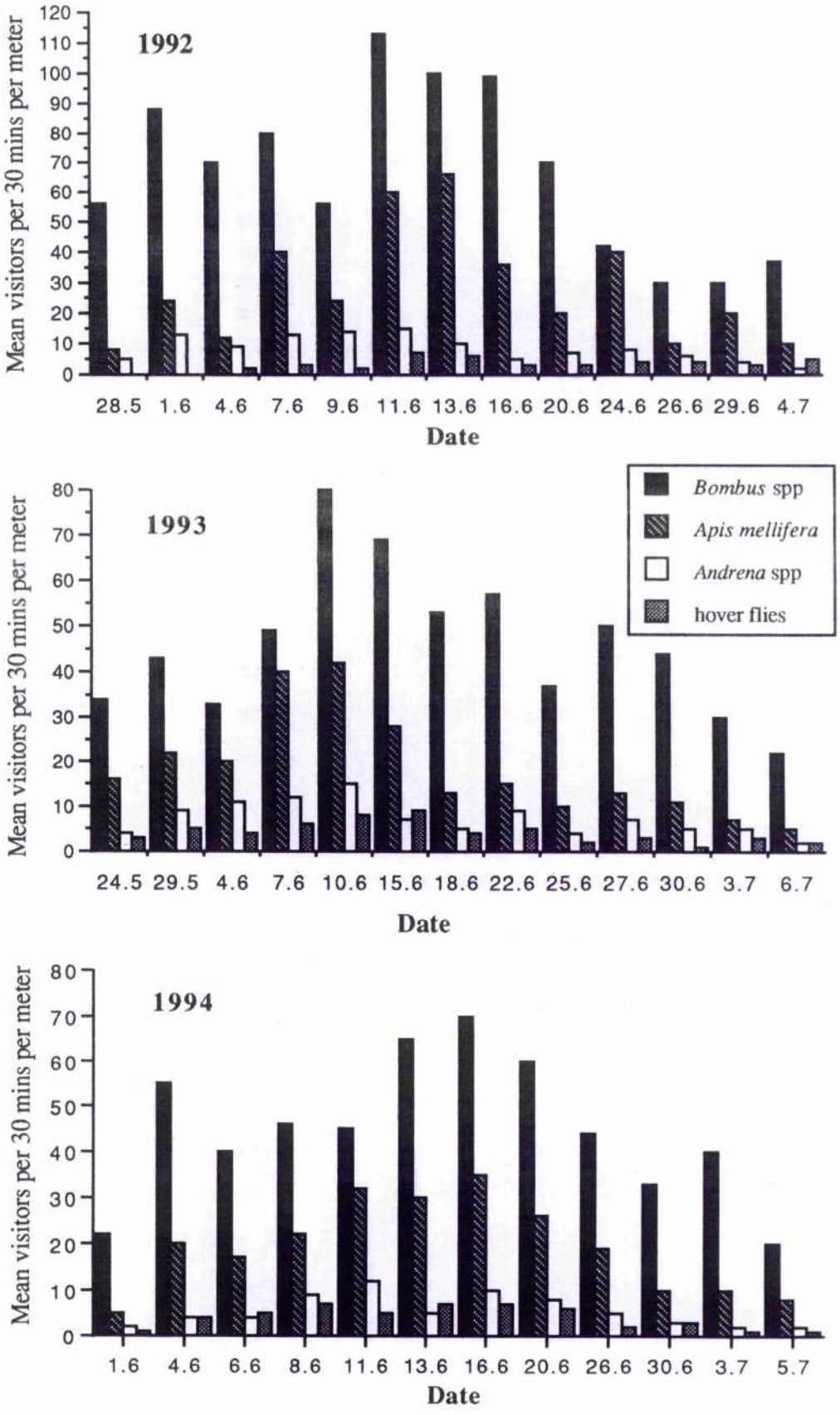

Fig.5.1. The seasonal abundance of insect visitors on Glen Moy flowers at SCRI throughout the three flowering seasons on all recording days.

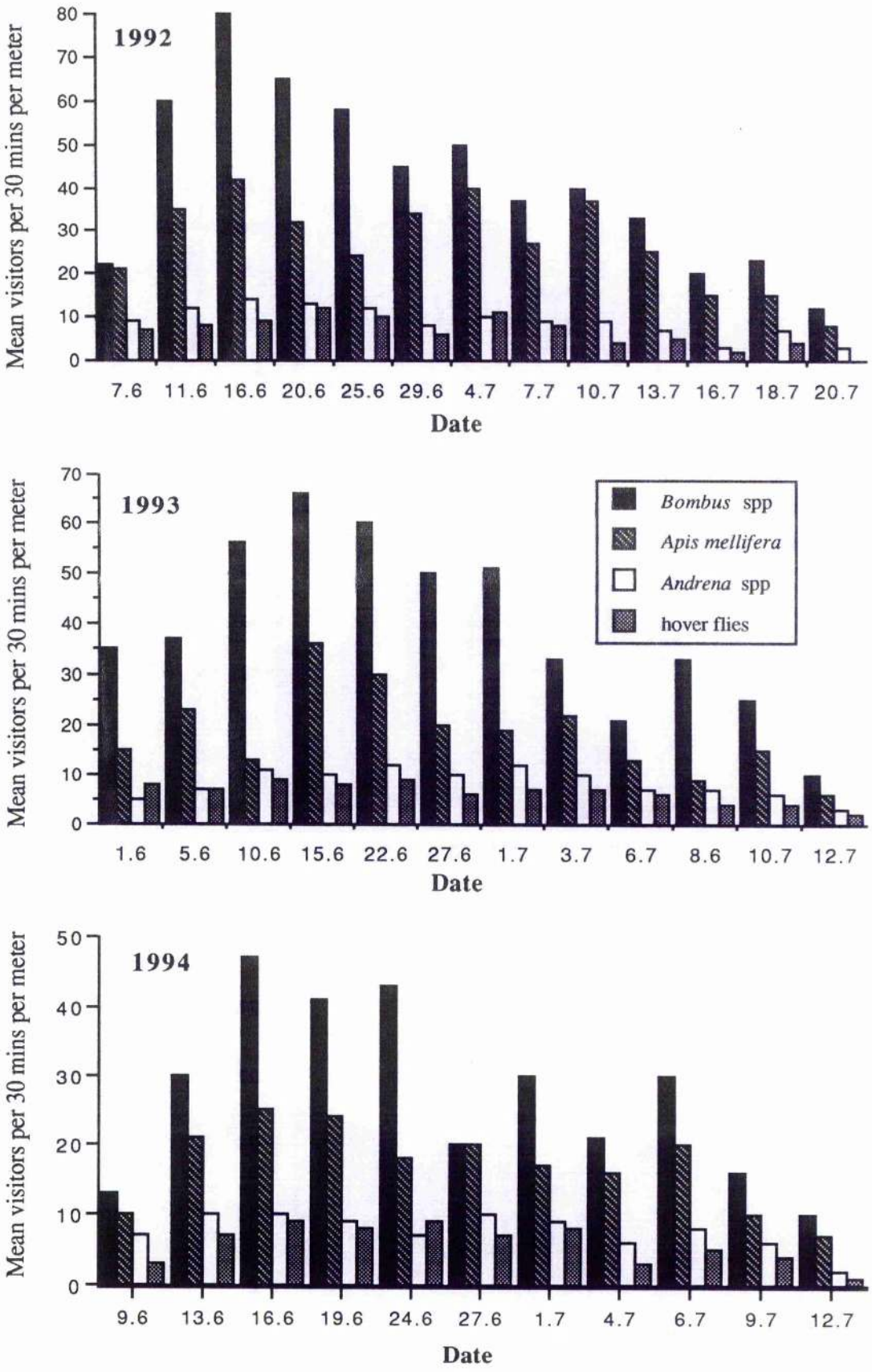

Fig. 5.2. The seasonal abundance of insect visitors on the Glen Prosen flowers at SCRI throughout the three seasons on all recording days.

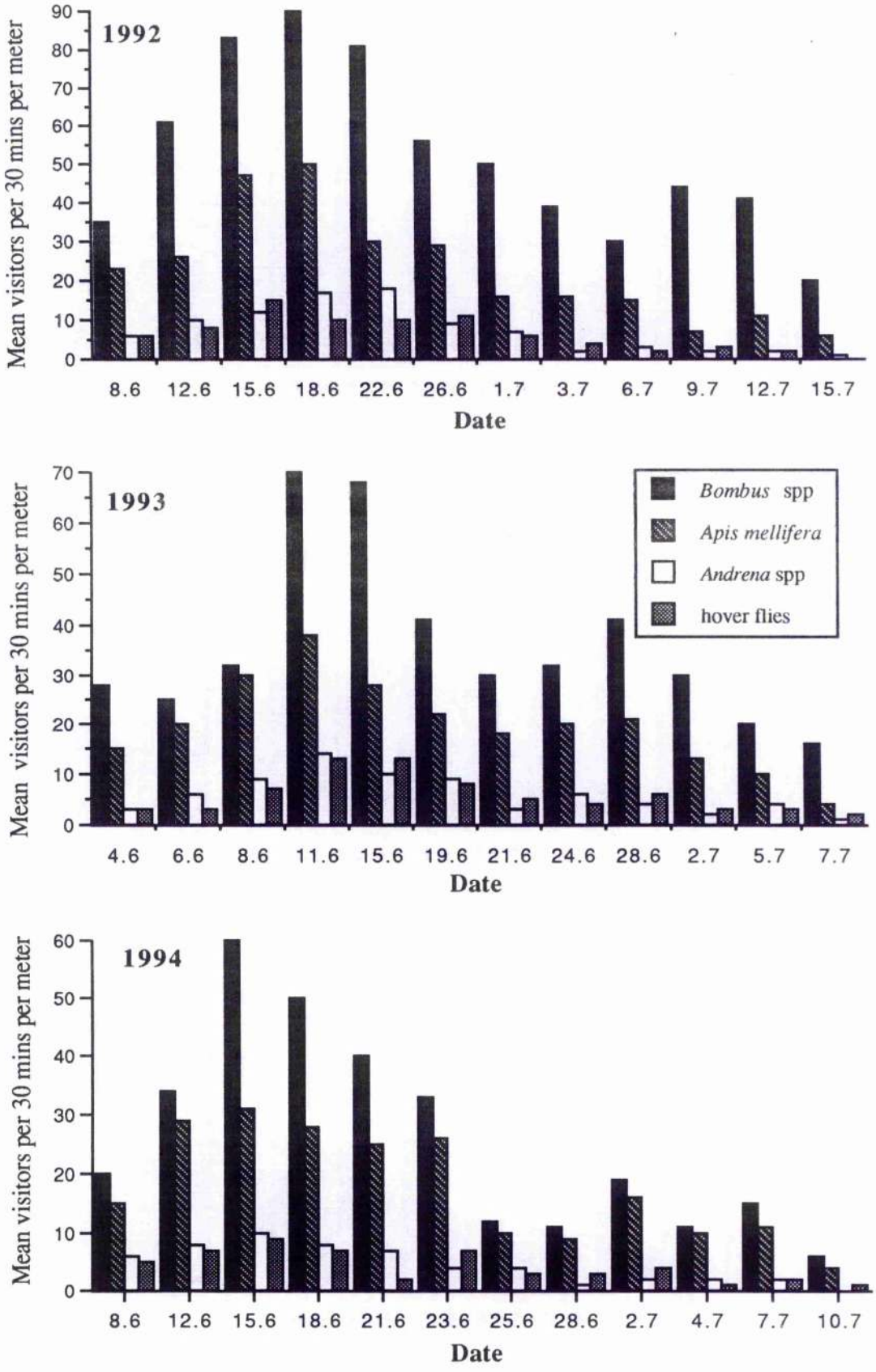

Fig.5.3. The seasonal abundance of insect visitors on wild raspberry flowers at Cameron reservoir throughout the three seasons on all recording days.

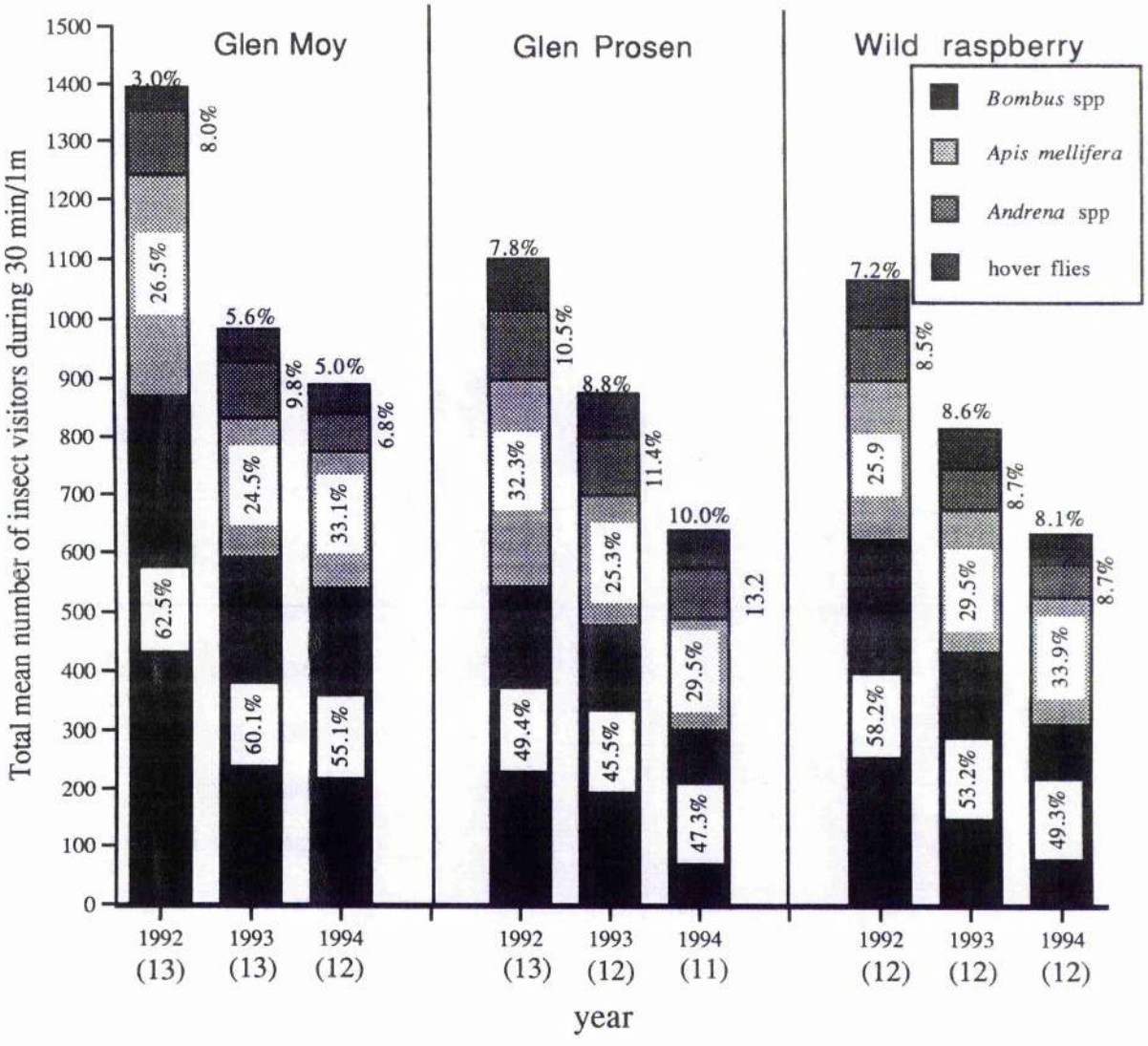

Fig. 5.4. A comparison of the percentage insect compositions which visited the three raspberry cultivars through the three different flowering seasons on all recording days. Numbers in brackets show the number of days of observation contributing to each total mean.

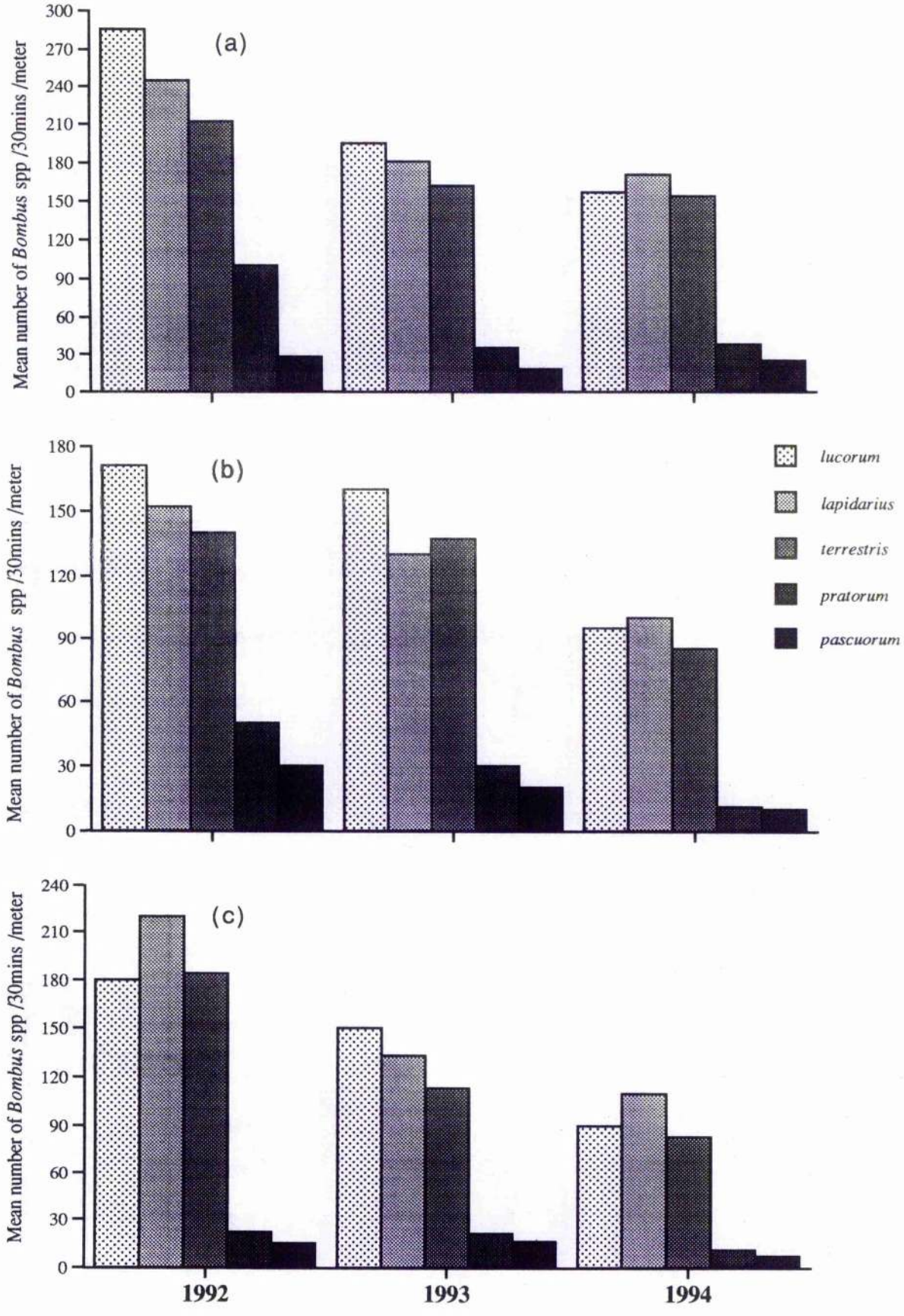

Fig.5.5. The pattern of *Bombus* species throughout the three different flowering seasons on (a) Glen Moy, (b) Glen Prosen and (c) wild raspberry flowers.

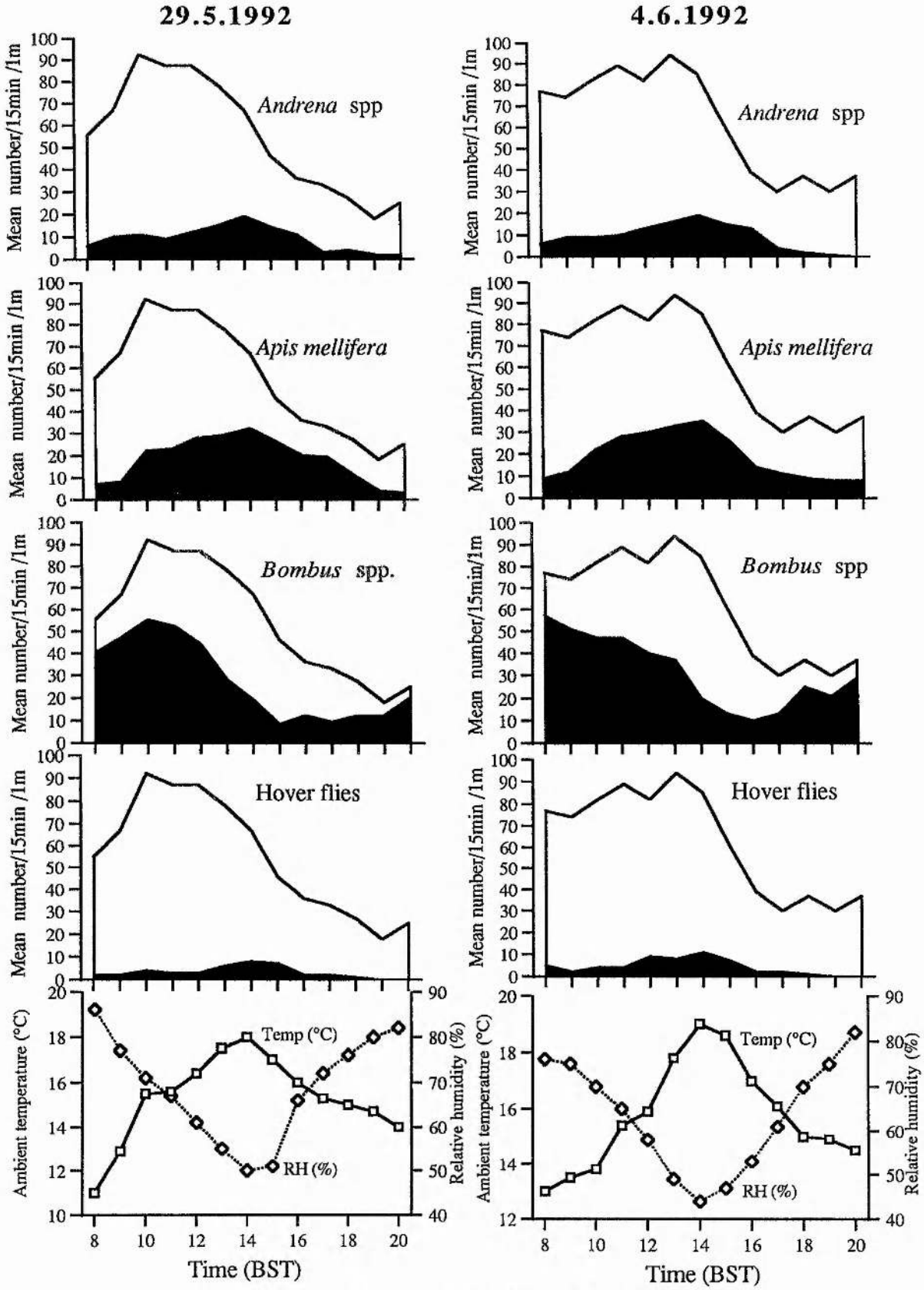

Fig.5.6. Temporal pattern of insect species visiting Glen Moy flowers on two different days (left: 29.5.1992 and right: 4.6.1992). in relation to the weather on the same day. The line shows the total visitors, and the shaded area shows the individual category of visitors in each case.

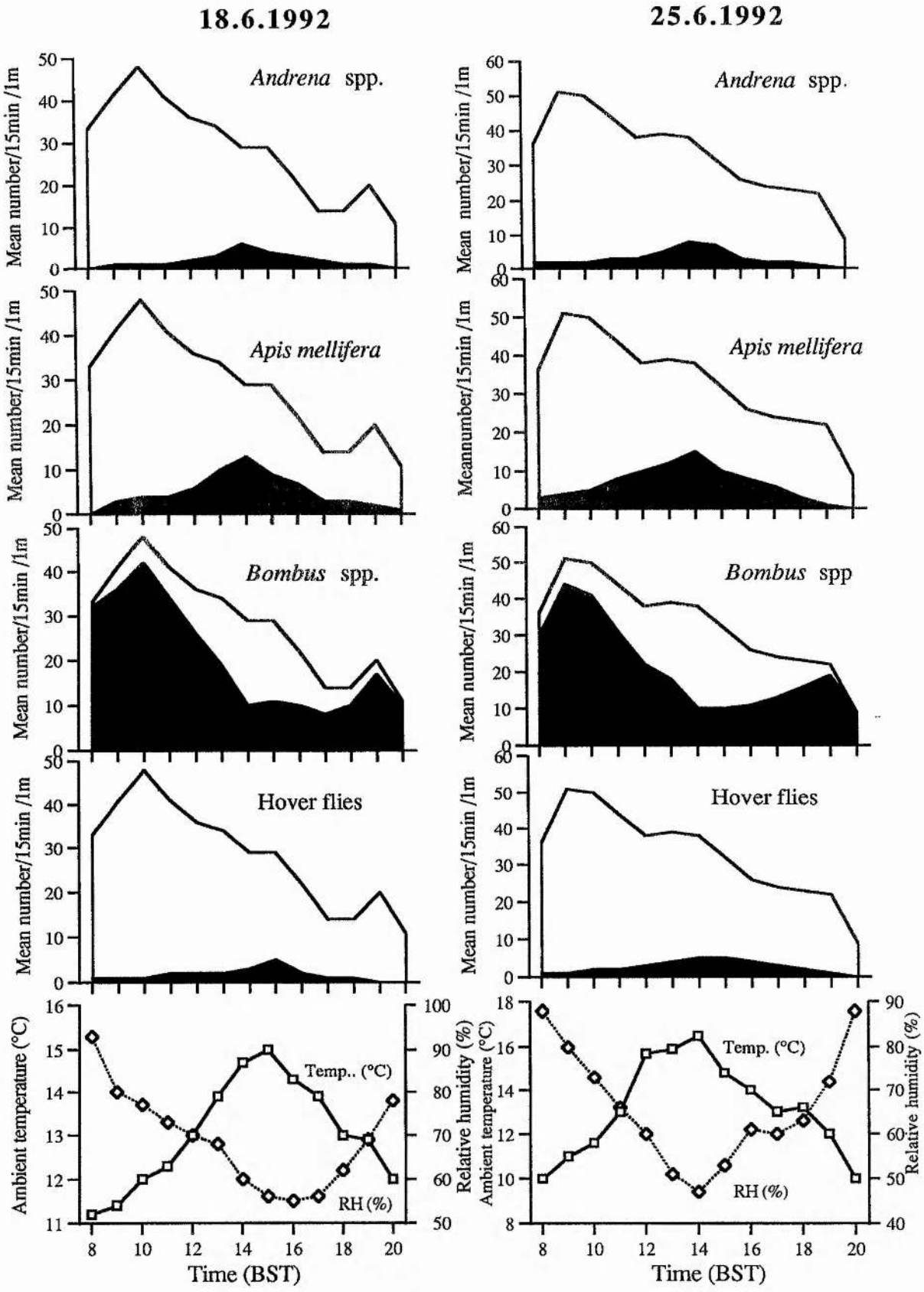

Fig.5.7. Temporal patterns of insects visiting Glen Prosen flowers on two different days (left:18.6.1992 and right: 25.6.1992), in relation to weather on the same days. The line shows the total visitors, and the shaded area shows the individual category of visitors in each case.

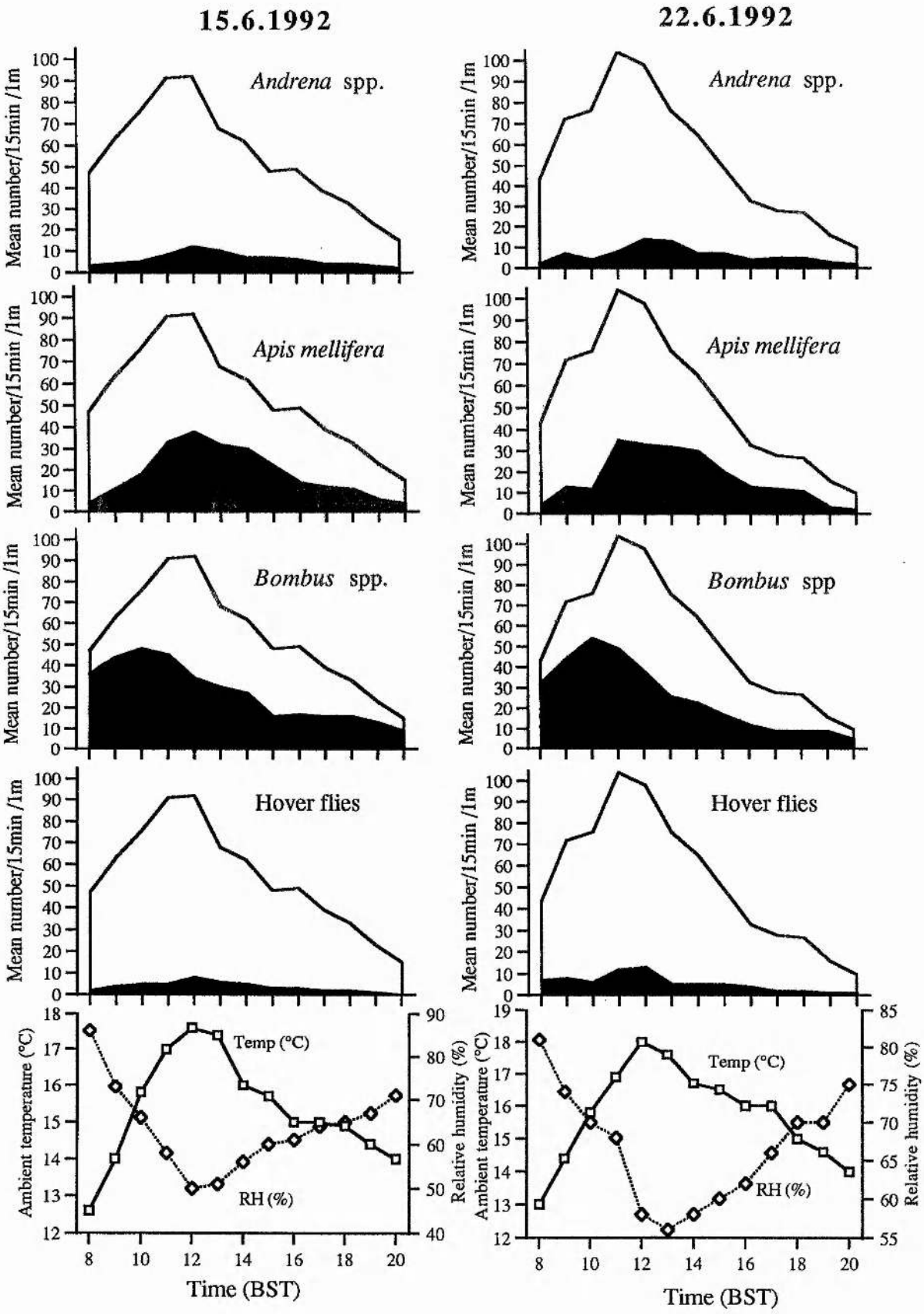

Fig.5.8. Temporal pattern of insect species visiting wild raspberry flowers on two different days (left:15.6.1992 and right: 22.6.1992) in relation to weather on the same day.The lines shows the total visitors, and the shaded area shows the the individual category of visitors in each case.

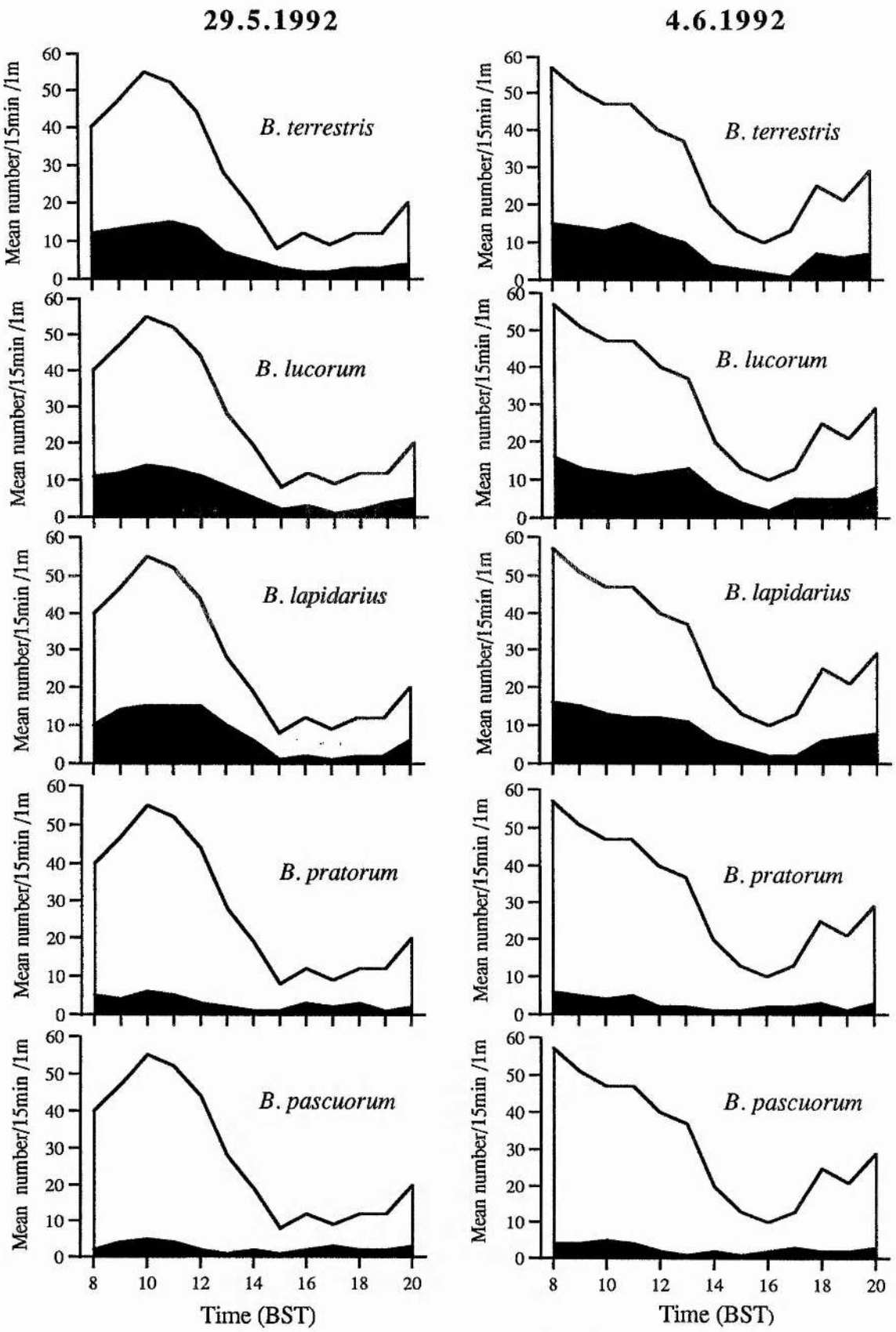

Fig. 5.9. Temporal pattern of *Bombus* species visiting Glen Moy flowers on two different days (left: 29.5.1992 and right:4.6.1992). Weather records as in fig 5.6. The line shows the total bumble bees, and the shaded area shows the individual bumble bee species.

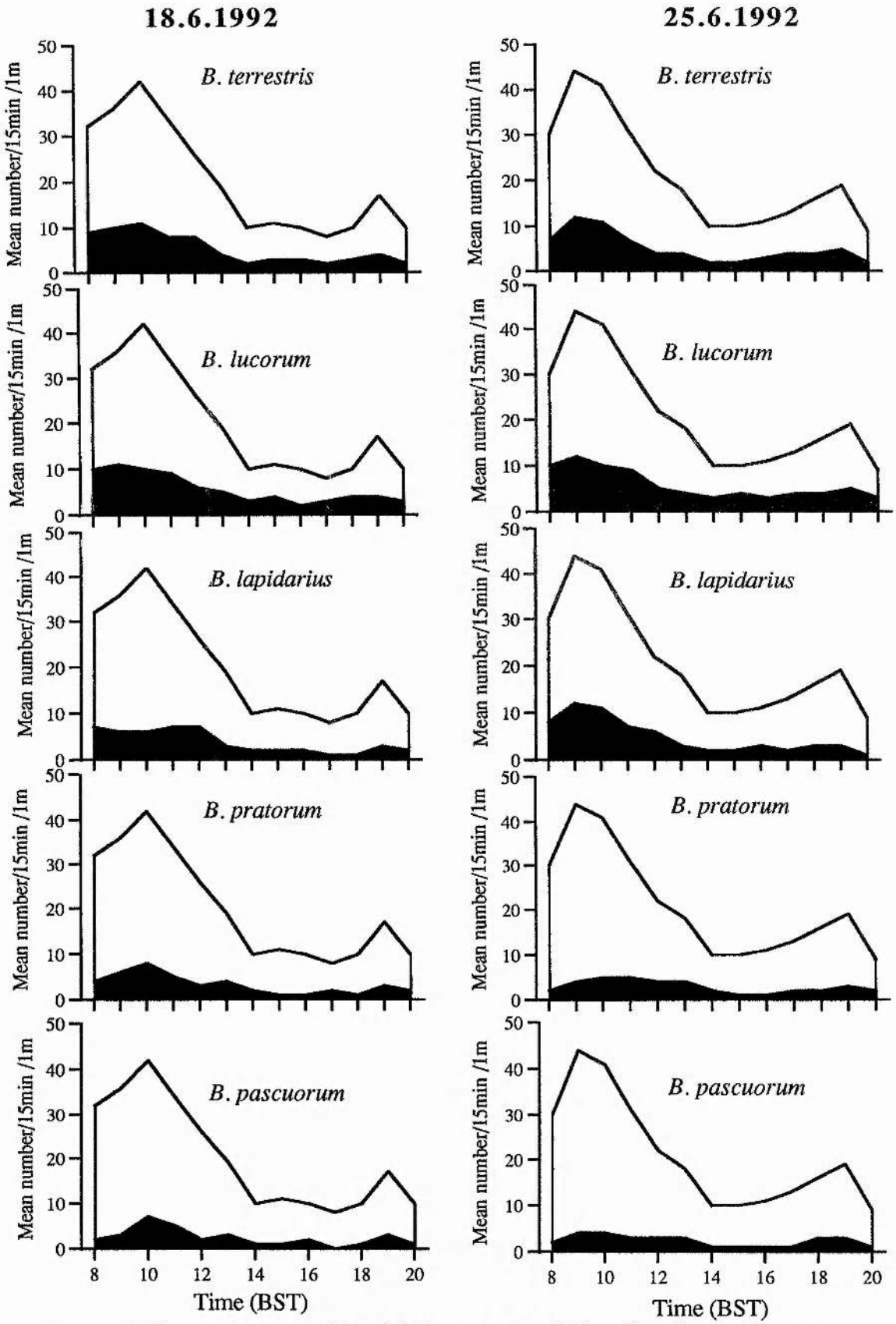

Fig. 5.10. Temporal pattern of bumble bees species visiting Glen Prosen flowers on two different days (left: 18.6.1992 and right: 25.6.1992). Weather records as in fig. 5.7. The line shows the total bumble bees, and the shaded area shows the individual bumble bee species.

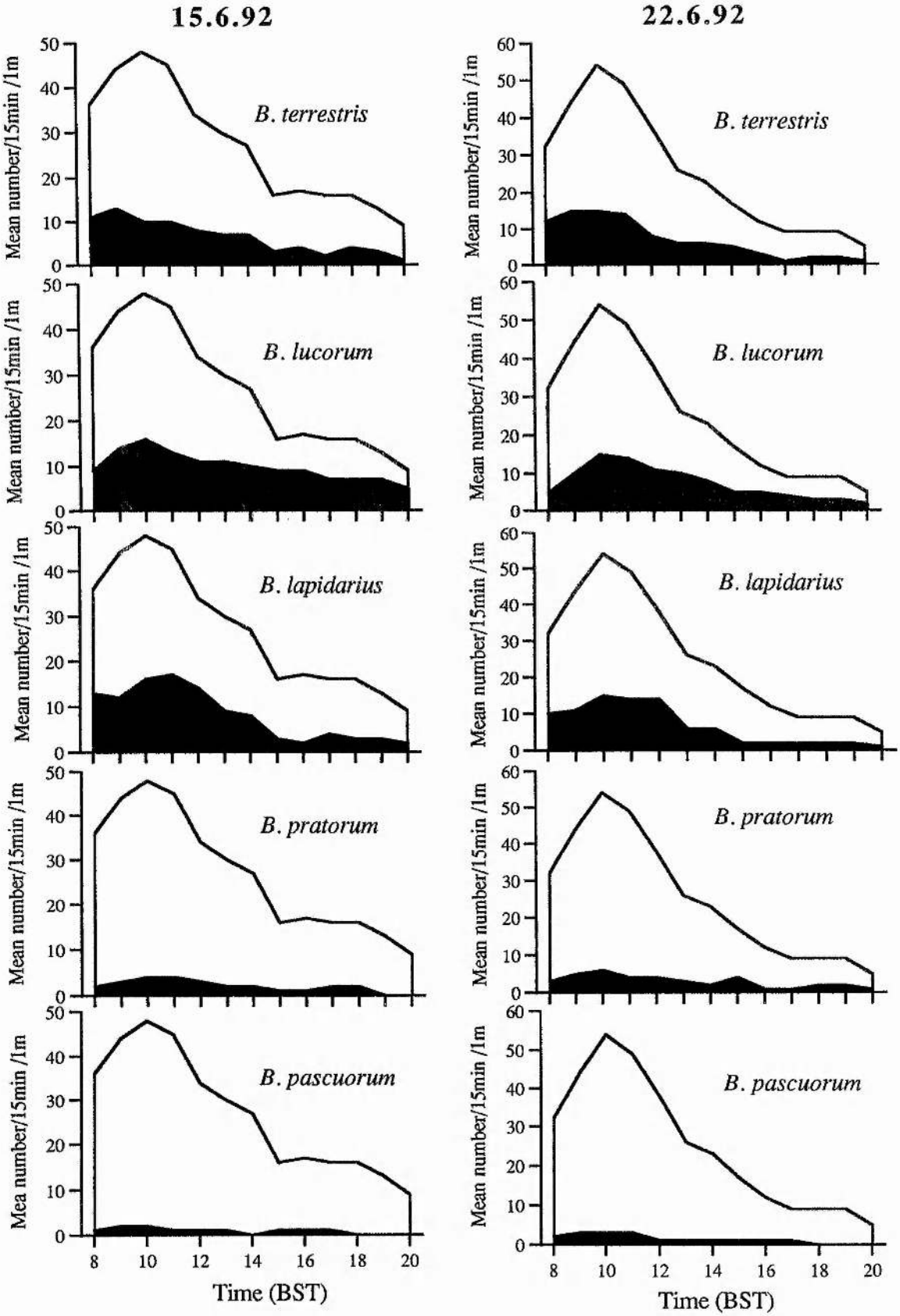

Fig. 5.11. Temporal pattern of *Bombus* species visiting wild raspberry flowers on two different days (left: 15.6.1992 and right: 22.6.1992). Weather records as in fig. 5.8. The line shows the total bumble bees, and the shaded area shows the individual bumble bee species.

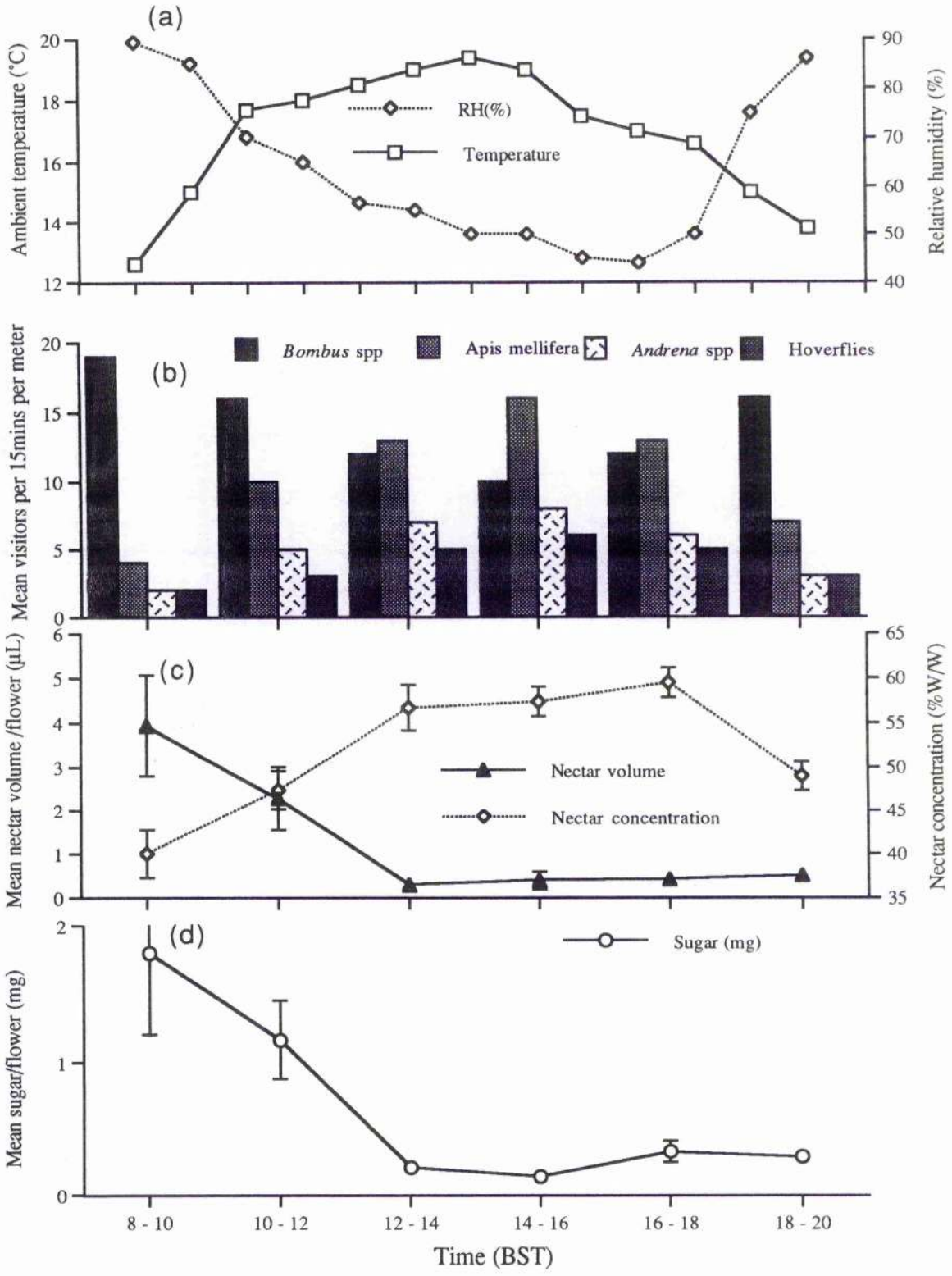

Fig.5.12. The pattern of insect visits to Glen Moy flowers on 5.6.1993. Insects were scored for 15 min at each interval over a 1 m patch (b). Prevailing microclimate (a), nectar availability (c) and sugar content (mg)(d) are also shown

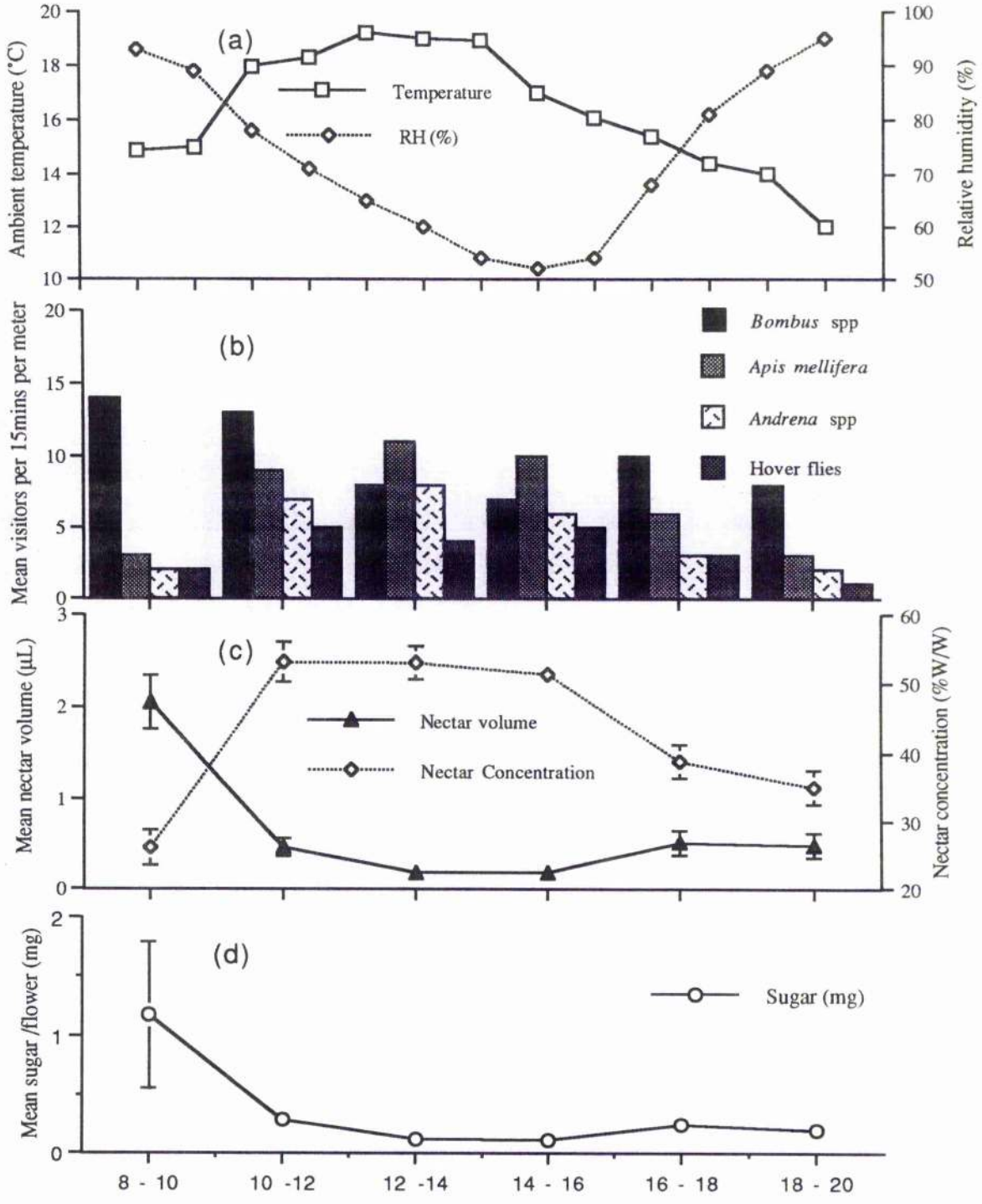

Fig.5.13. The pattern of insect visits to Glen Prosen flowers on 17.6.1993. Insects were scored for 15 min at each interval over a 1 m patch (b). Prevailing microclimate (a), nectar availability (c) and nectar content (mg) (d) are also shown.

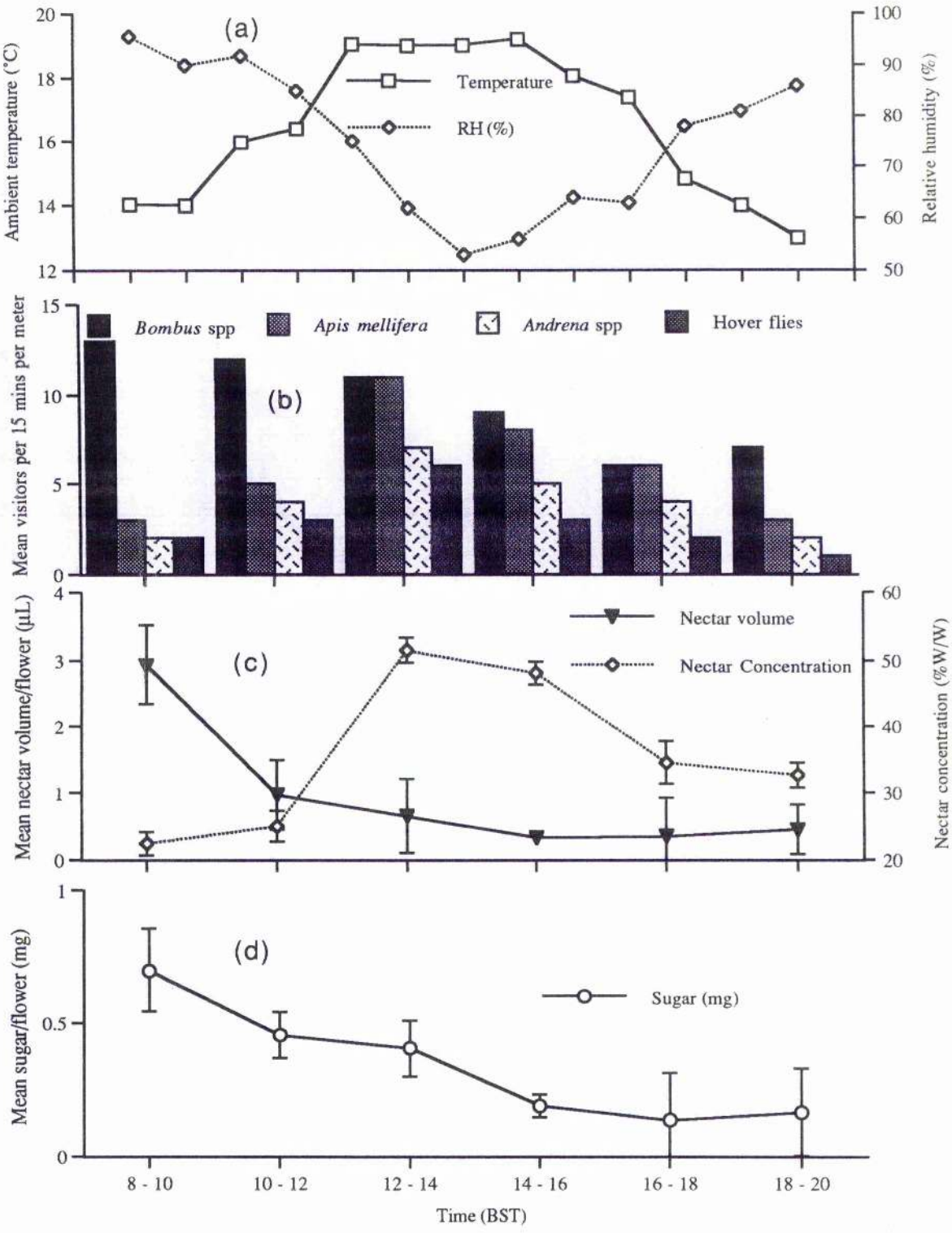

Fig. 5.14. The pattern of insect visits to wild raspberry flowers on 28.6.1993. Insects were scored for 15 min at each interval over a 1 m patch (b); prevailing microclimate (a), nectar availability (c) and sugar content (mg) (d) are also shown.

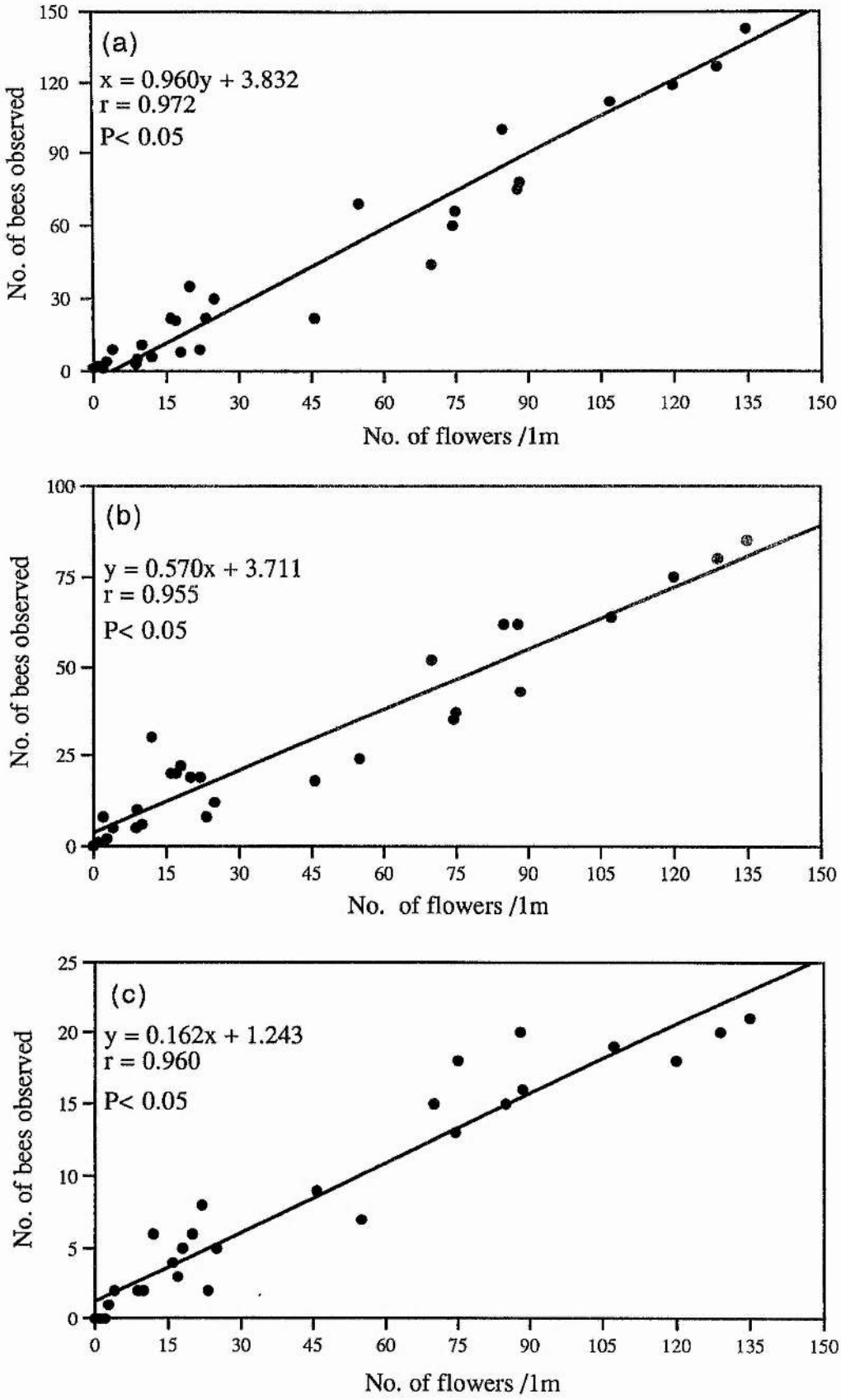

Fig. 5.15. The relationship between the number of Glen Moy flowers per meter and the number of (a) *Bombus* spp, (b) *Apis mellifera* and (c) *Andrena* spp foraging during 15 min. Regression lines are shown.

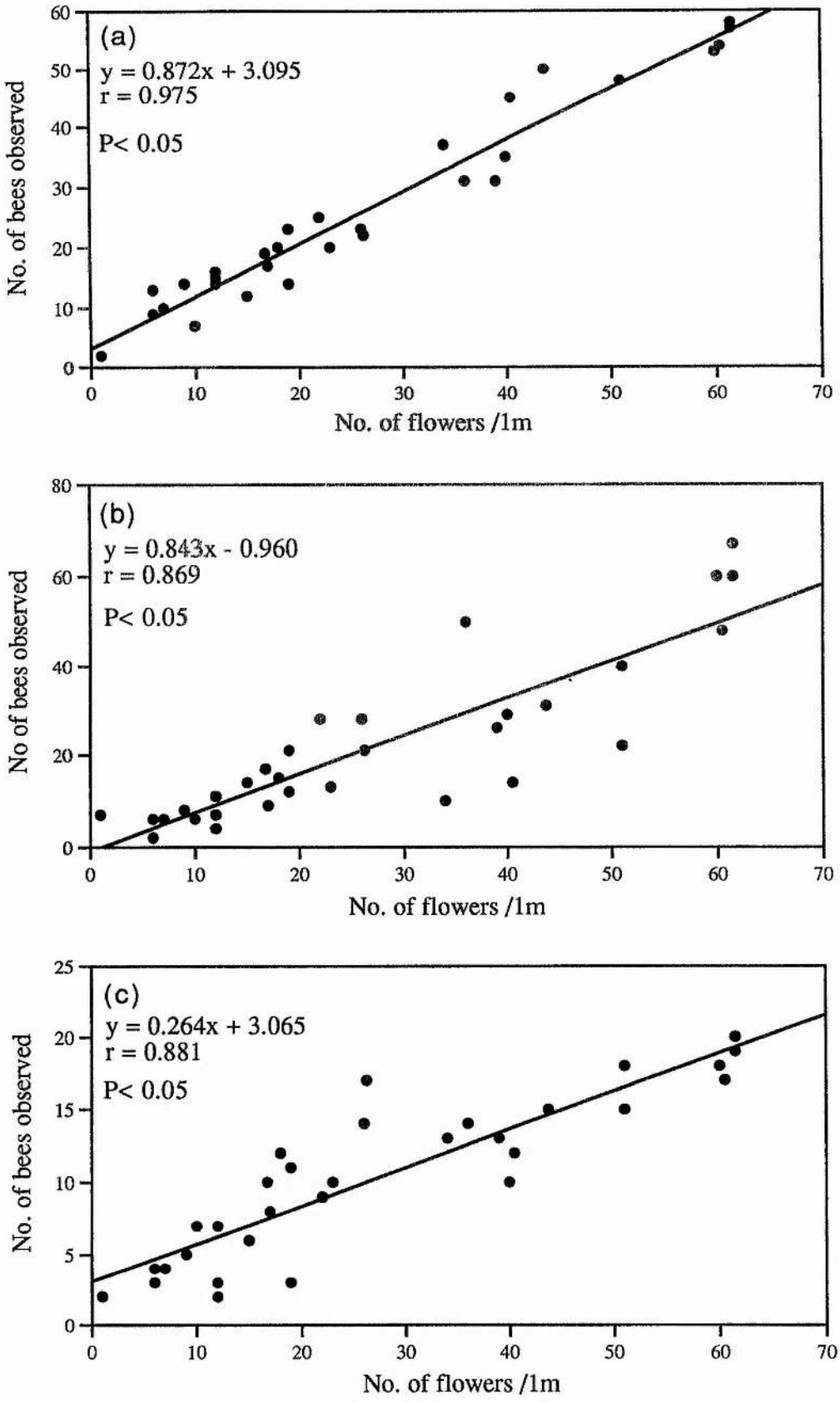

Fig. 5.16. The relationship between the number of Glen Prosen flowers per 1 metre and the number of (a) *Bombus* spp, (b) *Apis mellifera* and (c) *Andrena* spp foraging during 15 min. Regression lines are shown.

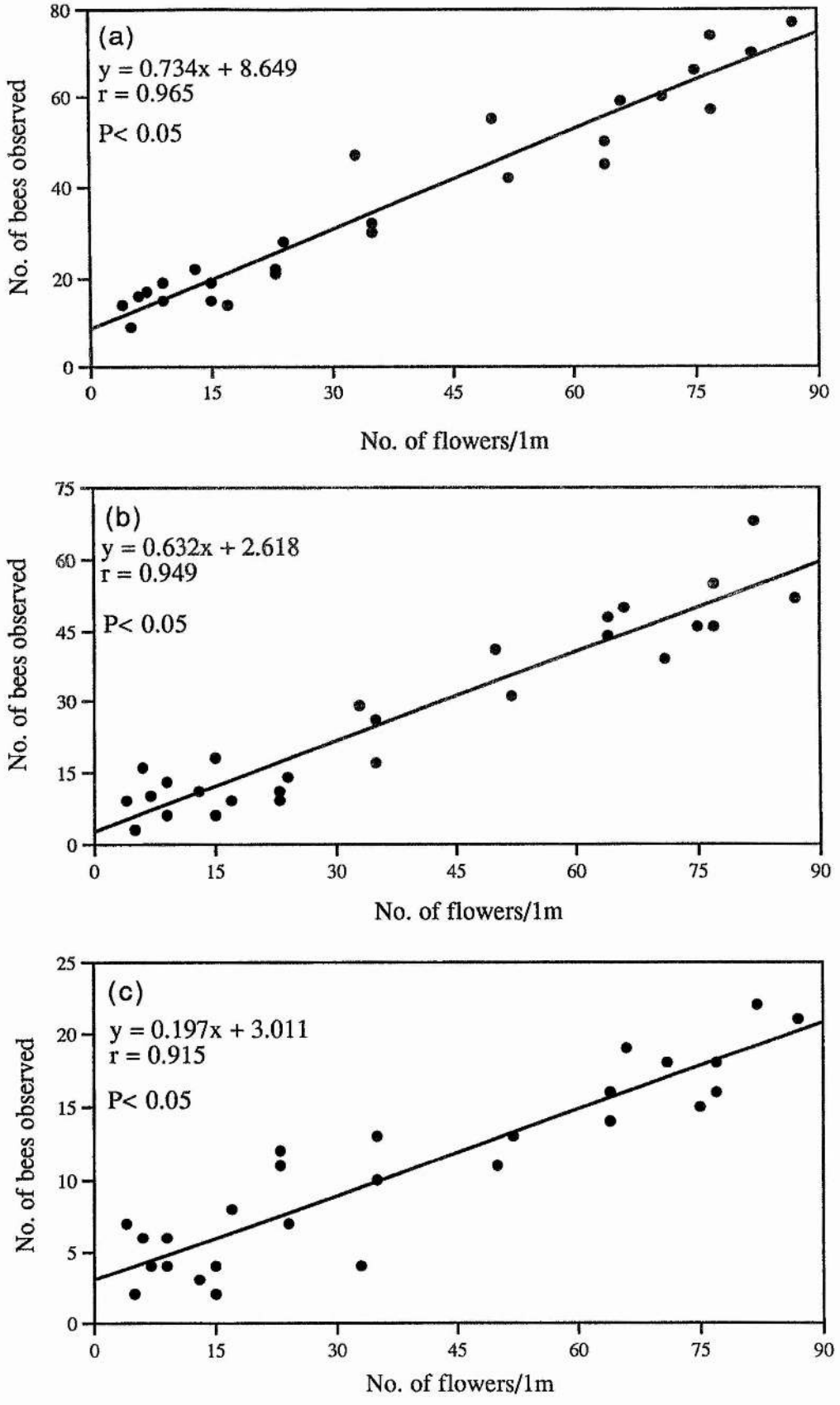

Fig. 5.17. The relastionship between number of wild raspberry flowers per 1 meter and the number of (a) *Bombus* spp, (b) *Apis mellifera* and (c) *Andrena* spp foraging during 15 min. Regression lines are shown.

Chapter 6
Foraging Behaviour

6.1. Introduction

6.2. Foraging Behaviour

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6.1. Introduction

Flowers provide two types of attractants for their visitors; primary, that satisfy demands for food (nectar and pollen), and secondary which serve as labels that start a direct or indirect reaction of the sensory system in visitors and would involve texture, form, colour and odour. Primary attractants serve as a reward for the visitor; however, they would be useless by themselves unless accompanied by secondary labels (Faegri & van der Pijl 1979).

The efficiency of pollination of any bee-pollinated crop must depend upon its profitability as a source of food for visitors. Without this reward and the accompanying sensory stimulation, the stimulus for the next response in the foraging sequence would be absent and the behaviour pattern broken (Doull 1961).

Insufficient transfer of pollen grains to receptive stigmas is one factor that has been proposed as a cause of low seed set in natural plant population (eg. Kevan 1972; Waser 1978). The contribution to a plant's reproductive success that each type of visitor makes is determined by both its visitation rate and its pollination effectiveness, i.e. the seed set resulting from a single visit (Beattie 1971b). Pollination effectiveness could be affected by the degree of floral preference of the visitors (Motten et al 1981).

The evaluation of the role of visitors in pollination of raspberry flowers can not be made on the basis of their abundance alone. The best estimates of visitors' pollination importance come from direct comparative studies of their pollination efficiency, either in pollen placement on stigmatic surfaces (e.g., Levin and Berube 1972; Ornduff 1975; Primack and Silander 1975; Willmer,

Bataw and Hughes 1994), or by direct measures of seed set after insect visitation (Motten *et al* 1981; Parker 1981).

Each insect visitor has certain characteristics which contribute to its function as an effective pollinator: its relative abundance on the host (which has been discussed in chapter 5), its pollen carrying capacity on its body, its degree of fidelity, and its foraging behaviour which, in conjunction with mechanical aspects of floral and inflorescence morphology, confer a specific pollination efficiency. All of these aspects have been considered to be of varying degrees of importance in analyses of pollination importance (Beattie 1971b; Bohart & Nye 1960).

This chapter represents analyses of each of the above components for visitors to the three raspberry cultivars (except the relative abundance of visitors which was discussed in chapter 5); from these data, I seek to estimate the relative pollination importance of visitor species in each type for raspberry cultivar.

6.2. Foraging behaviour

Bombus spp followed the same behaviour on cultivated and wild raspberries. The bigger individuals stood on the petals and smaller ones stood on stamens while they pushed their heads, and extended tongue, between the outer circles of stamens and central stigmas, down to the ring of nectary tissue lining the receptacular cup. They then moved around the flower, following the ring of nectaries (cf. Gilbert 1983), and their head and body touched the stigmas throughout. Small bees (*Apis mellifera* and *Andrena*) and other small insects stood either on the petals or the anthers, with their heads between the stamens and stigmas in order to reach the nectaries, and they too tended to work around

the flower. The rotation of the insect around the flower presumably increased the accumulation of pollen grains on the body. All bees touched the anthers and got pollen on their bodies.

When pollen was collected, the bees pivoted quickly on the flower; some of them packed the pollen into their pollen basket, others cleaned it from their bodies and discarded it. Some honey bees not collecting pollen discarded the accumulated grains by brushing their body hairs with the legs before moving on.

If the flower was dehiscing, most of the insect's body, and its head in particular, become covered with white pollen grains, and this was especially true of *Andrena spp* and *Apis mellifera*. At the end of a day of intensive foraging when available nectar and pollen standing crops seemed low (see chapter 4), all bees were observed to behave rather differently. They forced the youngest flowers open by inserting their proboscis between the closed petals.

Many bees were seen to rest on the flowers or leaves and clean themselves before taking flight. In the case of *B. pascuorum*, the bee always flew away from the bushes when any other bee approached on adjacent flowers, even if it was a conspecific.

It was very easy to distinguish whether the insect was collecting pollen, nectar or both in the field, in particular for the bees. In the case of nectar collection the bees extend their tongues between the stamens and stigmas.

6.2.1. Nectar and pollen collection

The overall proportions of insect visitors observed foraging on raspberry flowers are shown in Table 6.1. Percentages are given as bees collecting pollen, nectar or both. Faegri & Van Pijil (1979) reported that bumble bees do

commonly collect both nectar and pollen during the same visit. But this was not true in my study for *Bombus* or for *Apis mellifera* and *Andrena* spp. For hover flies, the percentages collecting both nectar and pollen in the same visit was very low even in comparison with the bees. The data indicate that both the bees and the hover flies mainly collect nectar during foraging, on both cultivated and wild raspberry flowers.

In order to understand the foraging behaviour of the bees throughout the day, Table 6.2 shows the break down of different foraging activities against time. This indicates that only a few of the bees collected pollen or both nectar and pollen in the same visit during the morning, when nectar was abundant. Pollen collection was very low before 1000 h, but increased during the day, peaking between mid and late afternoon. The bees therefore collected both pollen and nectar in the same visit when nectar was more scarce in the flowers in the afternoons. But in terms of pollination these distinctions may not matter; the pollen grains adhere to the body of bees during all flower visits, whether for collecting nectar, pollen or both.

6.2.2. The timing of foraging bout

The data in Table 6.3a indicate that *Apis mellifera* spent longer times visiting the raspberry flowers than *Bombus* spp and *Andrena* in all the raspberry cultivars' flowers; especially when visiting wild raspberry flowers *Bombus* spp. handling time was higher on Glen Moy than Glen Prosen and wild raspberry, and this could be because the nectar is more abundant in Glen Moy flowers than the other cultivars (see chapter 4). All the bees spent least time visiting Glen Prosen flowers. Most of the data were collected between 0800-1200h, when nectar was most abundant, so that trends through a day cannot be analysed in detail.

In a parallel study, Willmer, Bataw and Hughes 1994 showed that individual species of *Bombus* showed slightly differing patterns when foraging on the variety Glen Clova. *B. lapidarius* (now a very common species in East Scotland) had the fastest handling time.

Apis mellifera spent the shortest times travelling between the raspberry flowers (Table 6.3b); means value were 4.35 secs for Glen Moy, 4.00 secs for Glen Prosen and 4.52 secs in wild. The travelling time in the case of *Andrena* spp. was also quite short, with *Bombus* spp taking significantly longer. This could be because bumble bees strongly select for younger flowers (especially *B. lapidarius* and *B. lucorum* see later), whereas *Apis mellifera* and *Andrena* spp. moved from flower to flower without regard for the flower age. They were therefore moving (often crawling rather than flying) to the nearest open flower, and the travel times between the flowers did not depend on the nature of the next flower chosen.

6.2.3. The foraging rate

The foraging rate on wild and cultivated raspberry was measured as the number of flowers visited by one bee for a one minute recording period on a defined patch. The results (Table 6.4) showed that the bumblebees visited more flowers in one minute in all raspberry cultivars than either *Apis mellifera* or *Andrena* species, thus indicating that their rapid handling time more than compensated for their slower travel time. All bees had the highest foraging rate on Glen Prosen, and this could be due to less handling time spent by visitors on Glen Prosen flowers as a result of less nectar offered, compared with other raspberry cultivars.

6.2.4 The selection of flower age

The results (Table 6.5) show that the bumble bees prefer to visit young rather than older flowers of both wild and cultivated raspberry. *Apis mellifera* and *Andrena* spp. appeared to be less selective between young and old flowers, and they tended to move from flower to flower on the same stem. In terms of preference, *Bombus* spp visited young flowers on average more than 80% of the time. In contrast, with *Apis* and *Andrena* spp only about 52 - 69% of flowers visited were young. The proportions of old and young flowers available during the experimental periods on the plant as a whole were also checked: in Glen Moy the young flowers were 42.7% and old flowers 57.3%, in Glen Prosen young flowers were 27.5% and old flowers were 72.5%, and in wild raspberry flowers the proportion of young flowers and old flowers were 30.3% and 69.7%. Thus bumble bees were being particularly selective for young flowers on Glen Prosen and wild raspberry where young flowers were relatively scarce.

In general, the visitors to Glen Moy showed more selectivity for younger flowers than they did for Glen Prosen and wild, and this may be due to the size of Glen Moy flowers (chapter 3), which were bigger than in the other cultivars. In addition the amount of nectar secreted during the immediate period of flowering by Glen Moy is greater than in both Glen Prosen and wild raspberry and this could serve to attract the bees.

6.2.5. Pollen load

6.2.5.1. Insect borne pollen

The flower constancies of bees to Glen Moy, Glen Prosen and wild raspberry flowers were estimated by examining the pollen grains carried on the bodies of *Bombus* spp., *Apis mellifera* and *Andrena* which visited the three

cultivars of raspberry flowers; and then comparing the percentages of *Rubus* pollen grains on their bodies, in relation to the % of pollen from other surrounding plants. This excluded the corbicula pollen, and thus only assessed that body pollen directly available for pollination, (though Davis (1992) indicates that pollen in the tibial baskets of bees may also contribute to fertilisation).

No attempt was made to identify the pollen grains since this would have been very difficult because of the similarity of pollen grain morphological features. The pollen was classified as *Rubus* pollen and unknown (Tables 6.6, 6.7 and 6.8).

In general the insects collected larger numbers of *Rubus* pollen grains than pollens from the surrounding plants. On average bumble bees had substantially more pollen on their bodies (range of means was 290 - 617 pollen grain (varying between species and cultivars)) than did honey bees (226 - 286 pollen grain (varies between cultivars)) or *Andrena* species (range of means was 170 - 236 (varies between cultivars)). Again the larger species of *Bombus*, *Bombus lapidarius*, *B. lucorum* and *B. terrestris* were most effective among the bumble bees, carrying more pollen (range of means was 318 - 617 pollen grains) compared to *B. pratorum* and *B. pascuorum* whose bodies carried less pollen (range of means was 290 - 300 pollen grains; although this is still more than *Apis* and *Andrena* when comparing for the same plant).

The data indicate that the bees carried more *Rubus* pollen grains on their bodies when they were foraging on cultivated raspberry than when visiting wild raspberry; this may be because the wild raspberry produces less pollen than cultivated raspberry (see Table 3.3).

An approximation of the flower fidelity of *Bombus* spp, *Apis mellifera* and *Andrena* species to raspberry flowers can be obtained by examining the percentages of *Rubus* pollen in relation to the pollen from other plants. *Apis mellifera* shows high fidelity at all the raspberry cultivars; it is more likely to visit only raspberry flowers, and so is virtually monolectic on any one foraging trip. On any one trip, 88 - 95% of the total number of pollen grains carried by *Apis* were *Rubus* in all the three cultivars (95% Glen Moy, 93% Glen Prosen and 88.5% in wild raspberry flowers).

Different *Bombus* species showed differences in the mean percentages of *Rubus* pollen carried and this varied across the three cultivars: 89 - 90% of total pollen grains in Glen Moy being *Rubus*; 77 - 84% in the case of Glen Prosen; and 69 - 71% in wild raspberry flowers. The data indicate that *Bombus* species were more constant on cultivated raspberry than on wild.

B. terrestris, *B. lucorum* and *B. lapidarius* showed a higher fidelity at the cultivated raspberry than other *Bombus* species did; *B. lucorum* and *B. lapidarius* also showed higher fidelity than other *Bombus* species at wild raspberry.

Andrena species showed relatively little variation in percentages of *Rubus* pollen grains carried on their bodies for different cultivars (81.2% in Glen Moy, 78.6% in Glen Prosen and 76.9% in the case of wild raspberry).

The tables show that percentages of bees with non-*Rubus* pollen present varied between the different species of raspberry visitors and between the three cultivars. The percentages of *Apis* individuals captured with non-*Rubus* pollen were very low, and almost the same in the two cultivated raspberries (7.1% in Glen Moy and 7.7% in Glen Prosen flowers) but higher at 13.3% in wild flowers. 10 - 20% of all *Andrena* bore other pollen types. However 15 - 43% of

all *Bombus* (varies with different species and cultivars) had at least some non-*Rubus* pollen on their bodies, indicating a reduced tendency to be monolectic within trips.

The percentage of non-*Rubus* pollen grains was greater on wild than on cultivated raspberry visitors, and this may be at least partly due to the greater local diversity of flowers around the wild raspberry site.

Andrena showed fewer pollen grains carried on their bodies than *Apis* and *Bombus* species, and this could be due to their small body size compared with the other bees investigated. They also shows less fidelity in cultivated raspberry. Overall though, all these insect visitors showed a good degree of flower fidelity to raspberry.

6.2.5.2. Pollen on stigmas

The most direct method of evaluating visitor effectiveness is to examine the number of pollen grains transferred to a recipient stigma from a single visit. All visitors for which I obtained effectiveness data, *Bombus* spp, *Apis mellifera* and *Andrena* spp, can successfully transfer pollen to targeted stigmas. A successful visit is defined as one in which the pollen tube growth can be seen clearly; and after a bee visit most of the stigmas showed evidence of multiple tubes developing, indicating the effectiveness of the transfer of viable pollen.

Table 6.9. shows how much pollen the bees deposited on a single visit to a previously protected raspberry flower. Bumble bee species transferred more pollen to the stigmas of all cultivars on each visit that they made than either *Apis mellifera* or *Andrena* species did. For example on Glen Moy means between 39 - 54 pollen grains per stigma were recorded (varying with species), compared with a mean of 27 grains for *Apis mellifera* and 18 grains for *Andrena*

spp. Despite the fact that the andrenids are about one-third the size of honey bees, they deposited compatible pollen on the stigma nearly as often as the honey bees.

The bumble bee species showed differences in the number of pollen grains deposited; *Bombus terrestris* showed the highest numbers deposited on Glen Moy and Glen Prosen stigmas, with *Bombus lucorum* showing higher numbers deposited on wild raspberry stigmas. These figures are rather high compared to the total number of pollen grains carried on a bees' body (see 6.6.1); given that many stigmas on each flower each received this many pollen grains, this discrepancy may arise from the fact that the bees' pollen loads were probably substantially underestimated owing to losses during capture. The values for pollen deposition are also high compared to my estimates of pollen grains available per flower when gathered by a paint brush (section 3.3), since paint brush hairs are a poor substitute for the much-branched hairs of a bee. Another source of error was that pollen was probably shed copiously into the muslin bags during these trials. The results showing greater pollen transfer by *Bombus* species than by *Apis* are in accord with similar result on the related *Rubus fruticosus* (Yeboah Gyan and Woodell 1987), although the amounts of pollen deposited were very low when compared with *Rubus idaeus*.

Although I have not estimated the number of insect visits a flower must receive to be adequately pollinated, it is clear that one visit from any of the three groups of bees carried large numbers of pollen grains on to stigmas; and if we take in to account the fact that the raspberry flowers received many more than one visit during the flowers' life span, the targeted bees evidently play a key role in raspberry pollination.

6.3. Discussion

The relative value of particular visitors as pollinators on any crop cannot be judged just from their frequencies, and simplistic views have certainly tended in the past to over-value honey bees as crop pollinators (Batra 1992). Quantification of the relative effectiveness of pollinators is an inexact and complex issue (Heinrich and Raven 1972; Primack and Silander 1975; Motten *et al* 1981); it must take into account that there are a range of factors concerned with floral selectivity, constancy and ultimately the effectiveness of pollen carriage and transfer leading to correct pollen-tube growth (cf Davis 1992).

Flowers and inflorescences with a high reward yield are expected to be visited more than those with lower yield (Cohen & Shmida 1993). The results given here show that the older flowers were attracting fewer pollinators than the young ones; the data thus appear to support the expectation of optimal foraging theory which would predict that the bumble bees should be most selective of flowers with highest rewards available. *Apis* and *Andrena* showed less selection for young flowers (which had both pollen and nectar available, whereas medium and old flowers only offered nectar) and they moved from flower to flower without regard for the age of the flower. This could explain why *Apis* and *Andrena* carried less pollen grains on their bodies, which also suggests that the chance of pollination occurring would be less for them than with bumble bees. Although *Apis* had a larger handling time during their visits to raspberry flowers, the number of pollen grains carried on their bodies were low when compared with *Bombus* spp.

The results indicated that the three insect groups can transfer pollen to the stigmas of the three raspberry cultivars under study. The major difference between the three insect groups are in the number of flowers they visited per

minute, the number of pollen grains carried on their bodies, and the numbers of pollen grains they transferred to stigmas.

The best way to study flower constancy in bees is to analyse their pollen load. The bumble bees are usually less constant to one plant species than honey bees (Free and Butler 1959), although several observers have reported a strong flower constancy among bumble bees (Mosquin 1971). However in my study bumble bees were less constant to the raspberry plants, especially in wild raspberry where there is a locally diverse plant flora in the surrounding area.

Bombus spp spent more time searching for young raspberry flowers (Table 6.3b) but they showed higher foraging rates than did honey bees and *Andrena* spp. From all of the comparisons described in this chapter, I can conclude that at least in east Scotland *Bombus* species are substantially better at transferring pollen between raspberry flowers, and between patches of raspberry, than are honey bees or *Andrena*. The main reason for this is that they prefer/select younger flowers more effectively than honeybees and *Andrena* spp, and it is the young flowers from which pollen is available (chapter 3), and between which it can be effectively transferred. They also carry significantly more pollen on their bodies, and deposit significantly more pollen on stigmas per visit than other bees.

Table 6.1. Summary of visitors behaviour on cultivated and wild raspberry flowers on all recording days (% of individuals collecting)

	Glen Moy			Glen Prosen			Wild		
Insect	N.	P.	Both	N.	P.	Both	N.	P.	Both
B. pratorum	54.0	28.0	18.0	56.9	23.1	20.0	52.2	26.1	21.7
B. lucorum	67.4	20.4	12.2	63.5	26.4	10.1	56.0	24.5	19.5
B. lapidarius	54.1	39.3	19.7	59.5	28.2	12.3	63.0	26.5	10.5
B. terrestris	65.8	20.8	13.4	59.1	26.8	14.1	69.5	14.1	16.4
B. pascuorum	79.6	16.6	3.8	71.1	12.2	16.7	60.0	20.9	19.1
Andrena spp.	62.6	26.8	10.6	56.0	22.0	22.0	56.7	31.1	22.2
Apis mellifera	66.3	15.8	17.9	72.4	15.2	12.4	57.4	27.8	14.8
Hover fly	75.5	20.8	3.7	81.3	8.7	10.0	88.4	6.9	4.7

N = nectar only P = pollen only Both = nectar and pollen

Table 6.2. Percentage numbers of bees collecting nectar, pollen or both throughout the day

Insects	Time of day (hours BST)	Glen Moy			Glen Prosen			Wild		
		N	P	Both	N	P	Both	N	P	Both
Bombus spp	8 - 10	80	20	-	73	27	-	86	14	-
	10 - 12	75	20	5	76	34	-	90	10	-
	12 - 14	64	30	6	80	15	5	76	20	4
	14 - 16	50	35	15	60	31	9	61	20	19
	16 - 18	51	37	12	49	40	11	46	40	14
Apis mellifera	8 - 10	94	6	-	80	20	-	85	15	-
	10 - 12	80	16	4	70	15	15	75	22	3
	12 - 14	76	20	4	60	31	9	66	16	18
	14 - 16	50	36	14	60	20	20	70	19	11
	16 - 18	40	38	22	55	39	6	61	31	8
Andrena spp	8 - 10	70	30	-	84	16	-	86	11	3
	10 - 12	60	34	6	72	19	9	77	17	6
	12 - 14	59	36	5	60	36	4	71	26	3
	14 - 16	60	30	10	66	22	12	65	25	10
	16 - 18	50	36	14	52	33	15	55	28	17

N = nectar only P = pollen only Both = nectar and pollen

Table 6.3.

A.

Handling time of visitors in raspberry flowers (Mean sec. / flowers ± SE).

Pollinators	Glen Moy	Glen Prosen	Wild
Bombus spp.	8.32 ± 0.23	7.17 ± 0.28	7.50 ± 0.20
Apis mellifera	12.42 ± 0.91	10.49 ± 0.82	13.36 ± 0.41
Andrena spp.	8.26 ± 0.20	7.87 ± 0.43	10.73 ± 0.39

B.

Travelling time of visitors between Raspberry Flowers (Mean sec/flowers ± SE).

Pollinators	Glen Moy	Glen Prosen	Wild
Bombus spp.	5.56 ± 0.26	5.25 ± 0.25	5.32 ± 0.43
Apis mellifera	4.35 ± 0.19	4.00 ± 0.28	4.52 ± 0.19
Andrena spp.	4.81 ± 0.16	4.75 ± 0.26	4.62 ± 0.41

Table 6.4. The foraging rate (number of flowers visited by one bee) in cultivated and wild raspberry during one minute.(Mean numbers of flower ± SE).

Pollinator	Glen Moy	Glen Prosen	Wild
Bombus spp.	4.3 ± 0.6	4.8 ± 0.8	4.5 ± 0.6
Apis mellifera	3.6 ± 0.8	4.1 ± 0.6	4.0 ± 0.3
Andrena spp	3.4 ± 0.5	3.8 ± 0.5	3.6 ± 0.9

Table 6.5. Percentages of total number of visits to old and young flowers of wild and cultivated raspberries.

	Glen Moy		Glen Prosen		Wild	
Visitors	Young	Old	Young	Old	Young	Old
	n = 144		n = 160		n = 144	
Bombus spp.	76.8	23.2	85.5	14.5	81.0	19.0
Andrena spp.	62.0	38.0	65.4	34.6	69.6	30.4
Apis mellifera	52.0	48.0	55.5	45. 5	54.6	45.4

Young = the first stage of flowering

Old = medium and old flowers.

Table 6.6. The mean number (± SE), and proportions, of pollen grains on the bodies of insect visitors to Glen Moy.

Insect	Mean no. of pollen grains on body		% pollen on body of all individuals		Bees with non *Rubus* pollen (%)
	Rubus	Non-*Rubus*	*Rubus*	Non-*Rubus*	
B. terrestris (13)	508 ± 94	40 ± 33	90.9	9.1	15.4
B. pratorum (12)	367 ± 70	60 ± 26	86.4	13.6	25.0
B. lucorum (14)	477 ± 80	70 ± 26	87.1	12.9	28.6
B. pascuorum (9)	389 ± 104	75 ± 47	82.8	17.2	22.2
B. lapidarius (12)	617 ± 187	70 ± 37	89.2	10.8	25.0
Apis mellifera (14)	286 ± 44	14 ± 14	95.0	5.0	7.1
Andrena spp. (10)	236 ± 24	54 ± 28	81.2	18.8	10.0

Table 6.7. The mean number (± SE), and proportions, of pollen grains on the bodies of insect visitors to Glen Prosen

Insect	Mean no. of pollen grains on body		% pollen on body of all individuals		Bees with non *Rubus* pollen (%)
	Rubus	Non-*Rubus*	*Rubus*	Non-*Rubus*	
B. terrestris (12)	433 ± 84	85 ± 28	84.6	15.4	33.3
B. pratorum (11)	327 ± 46	99 ± 40	77.9	22.1	27.2
B. lucorum (13)	400 ± 57	69 ± 26	84.6	15.4	30.8
B. pascuorum (9)	356 ± 69	74 ± 33	82.5	17.5	33.3
B. lapidarius (9)	400 ± 72	95 ± 36	80.8	19.2	30.8
Apis mellifera (13)	246 ± 32	17 ± 15	93.7	06.3	7.7
Andrena spp. (10)	220 ± 20	60 ± 30	78.6	21.4	20.0

Table 6.8. The mean number (± SE), and proportions, of pollen grains on the bodies of insect visitors to wild raspberry

Insect	Mean no. of pollen grains on body		% pollen on body of all individuals		Bees with non *Rubus* pollen (%)
	Rubus	Non-*Rubus*	*Rubus*	Non-*Rubus*	
B. terrestris (12)	383 ± 29	116 ± 38	69.7	30.3	33.3
B. pratorum (13)	300 ± 43	92 ± 36	69.7	30.6	38.5
B. lucorum (14)	371 ± 65	85 ± 27	77.1	22.9	42.8
B. pascuorum(14)	290 ± 19	71± 33	70.5	29.5	35.7
B. lapidarius(11)	318 ± 29	90 ± 31	71.7	28.3	36.7
Apis mellifera(15)	226 ± 18	26 ± 18	88.5	11.5	13.3
Andrena spp. (15)	173 ± 18	40 ± 21	76.9	23.1	28.6

Table 6.9. The number of pollen grains carried to raspberry stigmas by the foraging insect (mean ± SE).

Insect	Glen Moy	Glen Prosen	Wild
B. terrestris	54.60 ± 0.6 (15)	51.60 ± 0.9 (11)	44.06 ± 1.2 (16)
B. lapidarius	44.22 ± 0.8 (19)	41.21 ± 0.7 (11)	41.02 ± 0.5 (11)
B. lucorum	49.31 ± 0.9 (11)	44.61 ± 0.9 (16)	48.07 ± 1.3 (18)
B. pascuroum	42.07 ± 0.7 (12)	39.20 ± 1.3 (12)	36.05 ± 1.9 (11)
B. pratorum	39.55 ± 1.3 (14)	35.40 ± 1.2 (14)	30.21 ± 0.8 (12)
Apis mellifera	27.08 ± 0.9 (14)	20.22 ± 0.6 (11)	19.94 ± 0.4 (12)
Andrena spp.	18.07 ± 0.9 (13)	22.70 ± 0.8 (13)	17.81 ± 0.7 (16)

The figures in bracket indicate the number of samples examined.

Chapter 7

Pollinator Flight Directionality

7.1. Introduction

Movements of pollen grains from specific sources are often difficult to determine directly, because of the size of pollen grains and lack of distinguishable pollen morphologies (Waddington 1981; Handel 1983). Ellstrand (1992) summarised four general approaches used to estimate gene flow by pollen. Most frequently, gene flow has been estimated by 1) measuring pollen dispersal from a point source either indirectly from pollinator foraging distance (e.g., Schmitt 1980) or from the dispersal of pollen analogues (Campbell & Waser 1989); or 2) by measuring gene dispersal from point or block sources. Pollen vectors have different efficiencies in transporting pollen (Handel 1983). Movement of pollen is controlled by their behaviour (Waser 1983), the size and pilosity of their bodies (Primack & Silander 1975), and the location of pollen grains on the insect body (Faegri & van der Pijl 1979; Proctor & Yeo 1973).

Pollinator movements and their directionality are thus an important controller of gene flow, but have been relatively infrequently investigated. Pyke (1978bc, 1979) investigated the movements of bumble bees between flowers within inflorescences of certain plants and he suggested that bumble bees, when exploiting a raceme, start with the lowest flowers which are richest in nectar. He also suggested that bumble bees forage in ways that minimise their energetic profit by starting with low flowers, moving from each flower to the closest vertically higher flower, and then moving to another inflorescence. Corbet *et al.* (1981) tested the extent to which Pyke's interpretation could be applied to other plants. They found that in the flowers of *Linaria vulgaris* there was less nectar sugar in the lower flowers than upper ones, while in *Scrophularia aquatica* they found no systematic relationship between the amount of nectar per flower and the position on the panicle. Also they reported that wasps (*Dolichovespula* and

Vespula spp) worked predominantly upwards when foraging for nectar on *Scrophularia aquatica*. The same was observed when bumble bees foraged for nectar on *Linaria vulgaris*. The only exception to these upwards movements was when *Bombus terrestris* foraged for nectar on *Linaria vulgaris*, where their subsequent movements were predominantly downwards. From these investigations they concluded that the pollinator's movement upwards or downwards may be dependent on the position the insects adopted during their visits.

Apart from the results presented in this thesis, little is known about the movements of bees visiting cultivated raspberry flowers and their role in transporting pollen grains among the flowers. In this chapter I will investigate whether the insect visitors to cultivated raspberry flowers show any preferences for landing on any part of the raspberry plant (starting point) when they first arrive, their movements between the individual flowers, and their role in transporting pollen grains.

7.2. Starting point

7.2.1. Landing site.

This experiment was conducted in the season 1994, in order to investigate the starting point of the insect visitors to the Glen Moy and Glen Prosen flowers when they forage for nectar. Wild raspberry was not used because of the difficulties of monitoring the insect visitors landing and movements. The observations were at different times through the day, and involved recording the landing position of the *Bombus* species, *Apis mellifera* and *Andrena* species on plants of the raspberry cultivars. At the same time that data were recorded, the nectar volumes in flowers of different positions were measured.

Table 7.1. shows the results from the observations of landing sites of *Bombus*, *Apis mellifera* and *Andrena* species on Glen Moy (a) and Glen Prosen (b) when they foraged for nectar. The results revealed that the landing sites of the insect visitors to Glen Moy and Glen Prosen included lower, middle and top flowers. However *Bombus* species, *Apis mellifera* and *Andrena* species all tended to commence foraging at the bottom of the raspberry canes, on both cultivars, even though flower number was lowest there. About 28% of Glen Moy flowers and 22% of Glen Prosen flowers were located in the lower part.

The amounts of nectar produced by the three different positions of flowers show significant differences between these levels, for Glen Moy (ANOVA: $df = 2$, $F = 35.94$, $P < 0.001$), and for Glen Prosen (ANOVA: $df = 2$, $F = 12.42$, $P < 0.001$).The average nectar per flower was lower for high flowers. Thus bees tend to land in the sites where reward per flower was highest.

7.2.2. Movement from the landing point.

In this experiment I investigated the movements of insects from the landing point and among the different sectors of the studied raspberry plant. The movements of *Bombus* species, *Apis mellifera* and *Andrena* species from the starting points on the two varieties of raspberry are summarised in Table 7.2. Because my investigation was mainly interested in the movements of bees between the different parts of the raspberry plant, I excluded some bees which concentrated their movements in one sector and moved away.

Most of the bee's movements were predominantly upwards when the bees landed on the bottom or middle sectors. Direct moves from either the bottom sector to top sector, or vice versa, were rare; (the percentages of bees moving from the top flowers to either middle or bottom was calculated from the total bees moved on the same plant after subtracting the bees that flew away from the plant). The percentages of bees moving from the middle sector to the

bottom was small compared with the total percentages of bees that moved upward. The result shows that most of the bees tend to move from one flower to a nearby vertically higher flower. The bees thus tend strongly to move vertically up each cane. This was the general pattern of insect movements within the canes of Glen Moy and Glen Prosen.

7.3. Pollinator flight directionality

7.3.1. Inter-floral movements

Data were obtained for 3550 bee flights performed by bumble bees, honey bees and *Andrena* species; 1747 on Glen Prosen and 1803 on Glen Moy. The distribution of the flights with reference to the base flight direction is illustrated in Fig. 7.1. It is evident that flights are not random. The percentages of directions of bees in relation to the eight base flight directions are presented in Fig 7.2ab. On Glen Moy 76.7% of the *Apis mellifera* movements were to the same row. i e. North or South. *Bombus* species showed about 64.2% and *Andrena* species about 71.6% of the total movements along the same row. On Glen Prosen about 74.9% of *Apis mellifera* movement were along the rows, while about 58.5% of *Bombus* and 71.6% of *Andrena* species moved along the same row.

Bees therefore show directionality in their foraging movements only in that they tend to fly straight ahead from flower to flower, within a row to the "left" moves occurring at about the same frequency as moves to the "right".

Many studies prove that pollinator directionality is important in increasing gene flow if pollen carry-over occurs (Levin & Kerster 1974). In my results the majority of bees tended to be flying in one direction (north - south, which represents the direction of all raspberry rows) and the pollinators tended to fly between adjacent flowers in the same row, probably because the inter-

plant distance within the row was much smaller than the inter-row distance. Consequently, when the pollinator changed plant, it usually selected the neighbour in the same row. Therefore mean gene flow between the plants in the same row is likely to be stronger than to the next row if any pollen carry over occurs.

7.3.2 Movements between Plots

All the observations and experiments which are described in the next paragraphs were conducted on the site mentioned in Chapter Two (fig 2.2).

The observations of the direct movements of individual bumble bees and honey bees between the centre (donor) plot and the different four arms (recipients) of cultivated raspberry flowers indicated that the movements showed highly significant differences between the movements to the four main direction (for *Apis* $x^2 = 100.8$, df = 3, $p < 0.01$, and *Bombus* $x^2 = 90.4$, df = 3, $p < 0.01$). Table 7.3. shows that most of the bumble bees and honey bees moved from the central plot to both north and south directions, and this could be because the rows in these two directions ran in the same direction as in the donor plot. Both *Bombus* species and *Apis mellifera* strongly tended to move in the same general directions as the rows ran.

7. 4. Pollen dispersal.

In order to investigate if the raspberry insect pollinators show any differences in transferring the pollen grains throughout the designed plot (fig 2.2), two experiments were conducted through the two different flowering seasons 1993 and 1994. These investigated the directions and distances which the raspberry's pollinators can transfer pollen grains by using fluorescent dye as a pollen analogue. The winds during the two experiments were light and variable in direction.

A - Experiment I

This experiment was conducted in the season 1993, in order to test whether the raspberry's pollinators can transfer the pollen grains equally to the four different directions. Fig 7.3 and Table 7.4. show highly significant differences in the number of flowers that received dye (pollen mimics) at different distances from the donor ($P < 0.001$). The number of recipient flowers marked with dye decreases with increase in distance. The number of flowers that received the dye was higher around the donor marked flowers (about 62.2% of the total number of marked flowers were in the first 10m around the donor in the different directions). Also the transfer of pollen showed significanct differences with the different directions. Most of the marked flowers were on rows running North - South, which is the same as the row direction of the donor plants.

B. Experiment II.

This experiment was conducted during the flowering season 1994 after the encouraging results I obtained in the previous season, in order to investigate whether *Bombus* species or *Apis mellifera* were responsible for transferring pollen grains in the different directions and to different distances.

Dye (pollen mimic) deposition on successive recipient flowers fluctuated dramatically (fig 7.4). Most of the surrounding flowers were marked with dye; in the case of *Bombus* species about 53.7% of the total marked flowers were the immediately surrounding flowers (within the first 10 m) in the different directions, while in the case of *Apis mellifera* about 50.2% of the total marked flowers were within the first 10 meters. The frequency of marked flowers declined on average with distance, indicated by negative regression coefficients in all the different directions for both species ($P < 0.05$). The frequency of dye (pollen mimic) transfer therefore decreases with increase in distance from

source in all the four directions. Dispersal occurred most frequently to nearby plants.

The dye particles transferred by *Bombus* species showed highly significant differences in deposition for different distances, and also showed highly significant differences in different directions (Table 7.5.). The highest frequencies of marked flowers were to the north, followed by the south. *Apis mellifera* also showed highly significant differences in depositing dye particles to different distances, but no significant differences were observed in transferring dye to the different directions (Table 7.6). In both cases there are no significant differences in dye deposited in different distances caused by different directions (i e. no interactions). The longest distance over which *Bombus* species were capable of dispersing the dye was 60m, while for *Apis mellifera* it was only 35 m.

Although these two experiments gave a good indication of how the raspberry pollinators can transfer dye in different directions and to different distances, it is important to note that powder may be an unreliable pollen analogue when we need to measure how many pollen grains are moved.

7.5. Discussion

Most of the bees tended to start off on each raspberry plant by landing on the bottom of the flowering area. Peak nectar abundance also occurred in the lowest flowers of each cane and nectar production decreased with increasing height of the flowers on a plant of both Glen Moy and Glen Prosen. Then the *Bombus*, *Apis mellifera* and *Andrena* species all tend strongly to move vertically upwards. Heinrich (1975b) suggested that such movements minimise revisitation of freshly emptied flowers. Benham (1969) suggested that the habit of bumblebees of foraging from the bottom to the top of inflorescences is a

response to, or is motivated by, the relatively higher nectar production of the lower flowers. Pyke (1979) stated that a bee could maximise its energetic profit by starting low, working upwards, and then moving to another inflorescence. Watching insect pollinators move among flowers on a plant and between plants in a population has yielded a tremendous amount of information about their foraging behaviour, and about the role of foraging movements in pollen flow among plant. Bees tend generally to fly from a flower to a near neighbour flower. There is a strong relationship between flight distance and mean nearest-neighbour distance between flowers (Levin & Kerster 1968; Pyke 1978c).

The majority of *Bombus*, *Apis mellifera* and *Andrena* movements between the plants of Glen Moy and Glen Prosen were between near-neighbour plants, a behaviour that has been reported in other plants (Pyke 1979). In raspberry this was presumably mainly because the flowers on adjacent plants on the same row were closer to each other than the flowers on the next rows, and the insects therefore had a closer view of them. The subsequent movements of insects during visits to raspberry flowers, which were mainly upwards and in one direction (North - South) require less time or energy than movement to another row or direction; the directionality of the pollinator therefore reduces foraging costs. Pyke (1978c) and Heinrich (1979) both found that changes in direction on successive flights are sometimes produced, probably influenced by reward obtained from flowers on an inflorescence.

Flight distance between raspberry flowers is a component of pollinator foraging behaviour most directly affecting plant gene dispersal. Honey bees tend to forage within rather sharply delineated areas at any one time, as do some bumble bees (Free 1993; Thomson *et al* 1982). In my studies, honey bees moved pollen mimics less far than did bumble bees. This could be as a result of the shorter foraging area of honey bees (Muller 1882, cited by Levin & Kerster 1974), which may be in turn be partly because honey bees are less likely to

"bypass" neighbouring plants than bumble bees, who may move further in flight either naturally or when disturbed. For example, most bumble bee flights on *Aquilegia caerulea* plants were either to closely neighbouring plants or were longer and involved by-passing near neighbour plants (Hodges and Russell 1981a). There is some indication of bimodality in *Bombus* species "distance moved" data in fig 7.4 to support this idea. Cresswell (pers. comm.) has also indicated that some bumble bees are especially likely to use this large range "bypass" flight pattern.

The pollen flow data presented in this chapter are based on the movement of fluorescent dye rather than pollen grains. Because dye tends to travel farther (Thomson *et al* 1986), reports here and elsewhere of restricted pollen flow distance based on dye flow (Price and Waser 1979) may well be overestimate.

Table 7.1. Percentages of insect visitors landing on a given flower sector of Glen Moy (a) and Glen Prosen (b). Nectar volume was also measured at the same time (the numbers between the brackets indicate the sample number).

(a)

	Bombus spp (1116)	*Apis mellifera* (295)	*Andrena* spp (145)	Nectar (μL)
Top sector	15.23%	13.43%	15.86%	1.4 ± 0.2 (61)
Middle sector	31.45%	29.85%	34.45%	1.8 ± 0.2 (59)
Lower sector	53.22%	56.71%	49.65%	3.7 ± 0.3 (62)

(b)

	Bombus spp (672)	*Apis mellifera* (236)	*Andrena* spp (104)	Nectar (μL)
Top sector	13.40%	13.56%	15.38%	1.0 ± 0.2 (60)
Middle sector	35.71%	30.51%	33.65%	1.2 ± 0.1 (55)
Lower sector	50.82%	55.93%	42.31%	2.0 ± 0.2 (52)

Table 7.2. The number of inter-plant movements of *Bombus* spp, *Apis mellifera* and *Andrena* spp. between the three different sectors on Glen Moy (a) and Glen Prosen (b). Percentages are indicated in the brackets. BM means movement of bees from bottom to middle sector .....etc.

(a)

Starting sector	Insect visitor	Sector moved to			Sample size (n)
		Bottom sector	Middle sector	Top sector	
Bottom	*Bombus* spp.		BM 420 (86.4)	BT 66 (13.6)	486
	Apis mellifera		BM 316 (90.5)	BT 33 (9.5)	349
	Andrena spp.		BM 100 (98.0)	BT 2 (2.0)	102
Middle	*Bombus* spp.	MB 30 (11.1)		MT 240 (88.9)	270
	Apis mellifera	MB 17 (12.6)		MT 120 (87.6)	137
	Andrena spp.	MB 6 (6.2)		MT 91 (93.8)	97
Top	*Bombus* spp.	TB 13 (34.2)	TM 25 (65.8)		38
	Apis mellifera	TB 12 (36.4)	TM 21 (63.6)		33
	Andrena spp.	TB 9 (45.0)	TM 11 (55.0)		20

(b)

Starting sector	Insect visitors	Sector moved to			Sample size (n)
		Bottom sector	Middle sector	Top sector	
Bottom	*Bombus* spp.		BM 301 (87.5)	BT 43 (12.5)	344
	Apis mellifera		BM 284 (92.8)	BT 22 (7.2)	306
	Andrena spp.		BM 88 (88.0)	BT 12 (12.0)	100
Middle	*Bombus* spp.	MB 14 (9.7)		MT 130 (90.3)	144
	Apis mellifera	MB 12 (11.2)		MT 89 (88.8)	101
	Andrena spp.	MB 6 (8.3)		MT 66 (91.7)	72
Top	*Bombus* spp.	TB 22 (43.1)	TM 29 (56.9)		51
	Apis mellifera	TB 10 (37.0)	TM 17 (63.0)		27
	Andrena spp.	TB 5 (41.7)	TM 7 (58.3)		12

Table 7.3. The number of flight movements of insect pollinators between raspberry plots to different directions.

	Movement direction			
Insect	North	South	East	West
Bombus spp.	316	387	193	211
Apis mellifera	283	265	141	123

Table 7.4. Analysis of covariance for frequency of dye particles carried by the different pollinators from the source (Glen Clova) to the recipient (Glen Lyon) in the different directions, from 1993 studies.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Distance	1	3309.06	3309.06	3309.06	78.21	0.001
Direction	3	316.91	382.90	127.63	3.02	0.039
Dir*Dis	3	106.71	106.71	35.57	0.84	0.478 ns
Error	48	2030.87	2030.87	42.31		
Total	55	5763.55				

Table 7.5. Analysis of covariance for frequency of dye particles carried by *Bombus* species from the source (Glen Clova) to the recipient (Glen Lyon) in the different directions, from 1994 studies.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Distanc	1	310.003	197.97	197.966	25.68	0.001
Direction	3	194.370	136.11	45.370	5.88	0.001
Dir*Distance	3	45.088	45.088	15.029	1.95	0.127
Error	92	709.289	709.28	7.710		
Total	99	1258.750				

Table 7.6. Analysis of covariance for frequency of dye particles carried by *Apis melliferra* from the source (Glen Clova) to the recipient (Glen Lyon) in the different directions, from 1994 studies.

Source	DF	Seq SS	Adj SS	Adj MS	F	P
Distance	1	101.263	74.876	74.876	26.27	0.000
Direction	3	33.447	20.067	6.689	2.35	0.078
Dir*Dist	3	11.786	11.786	3.929	1.38	0.254
Error	92	262.254	262.254	2.851		
Total	99	408.750				

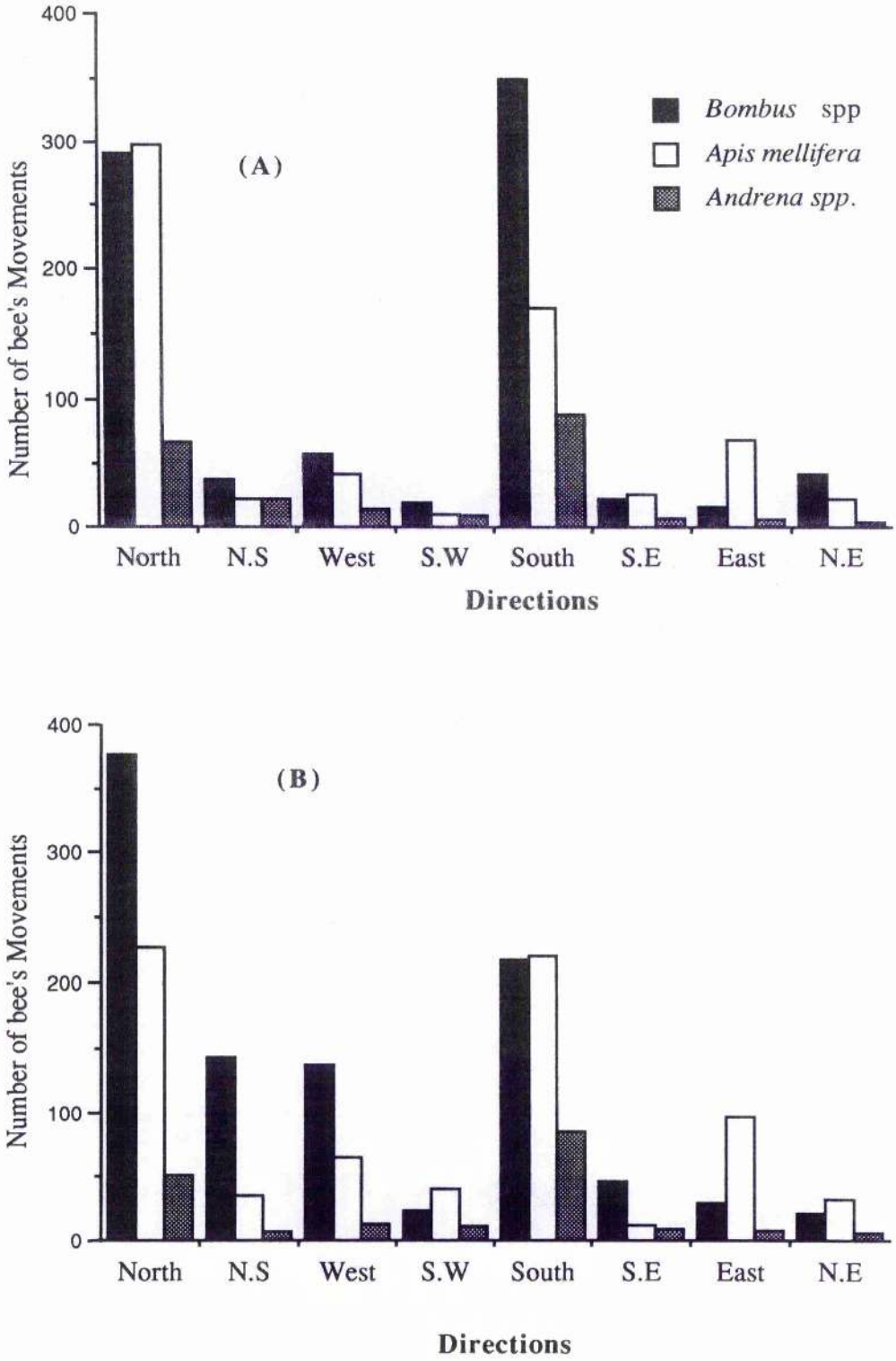

Fig. 7.1. The number of visitor's movements through the flowers of Glen Moy (a) and Glen Prosen (b), showing the number of each bee species movements to the different directions. North and South are the directions in which the rows of canes run.

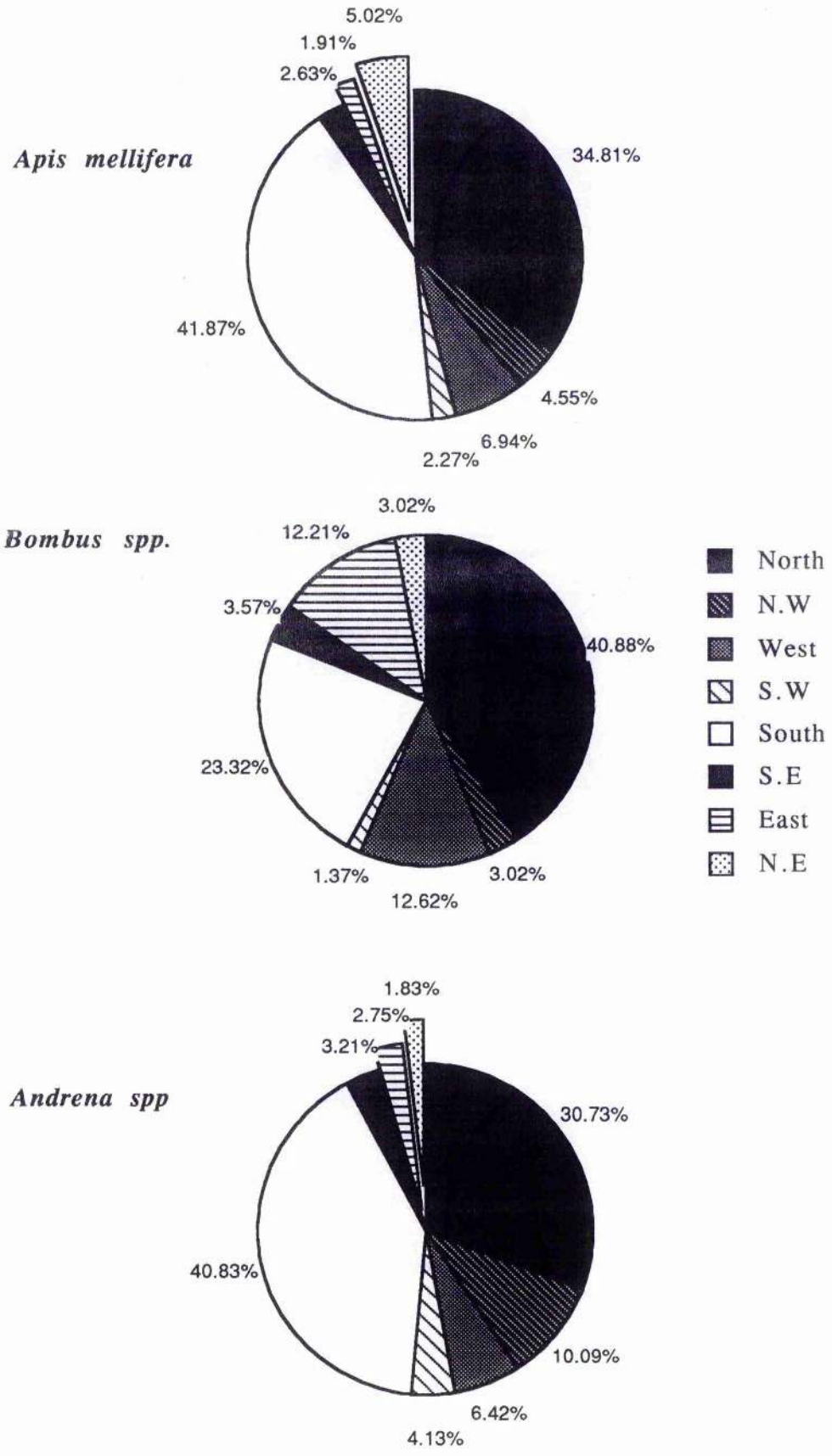

Fig. 7.2a. The percentages of bee's movements to different directions on Glen Moy flowers.The raspberry rows run from north to south, while the E -W direction represents the movements to the adjacent rows.

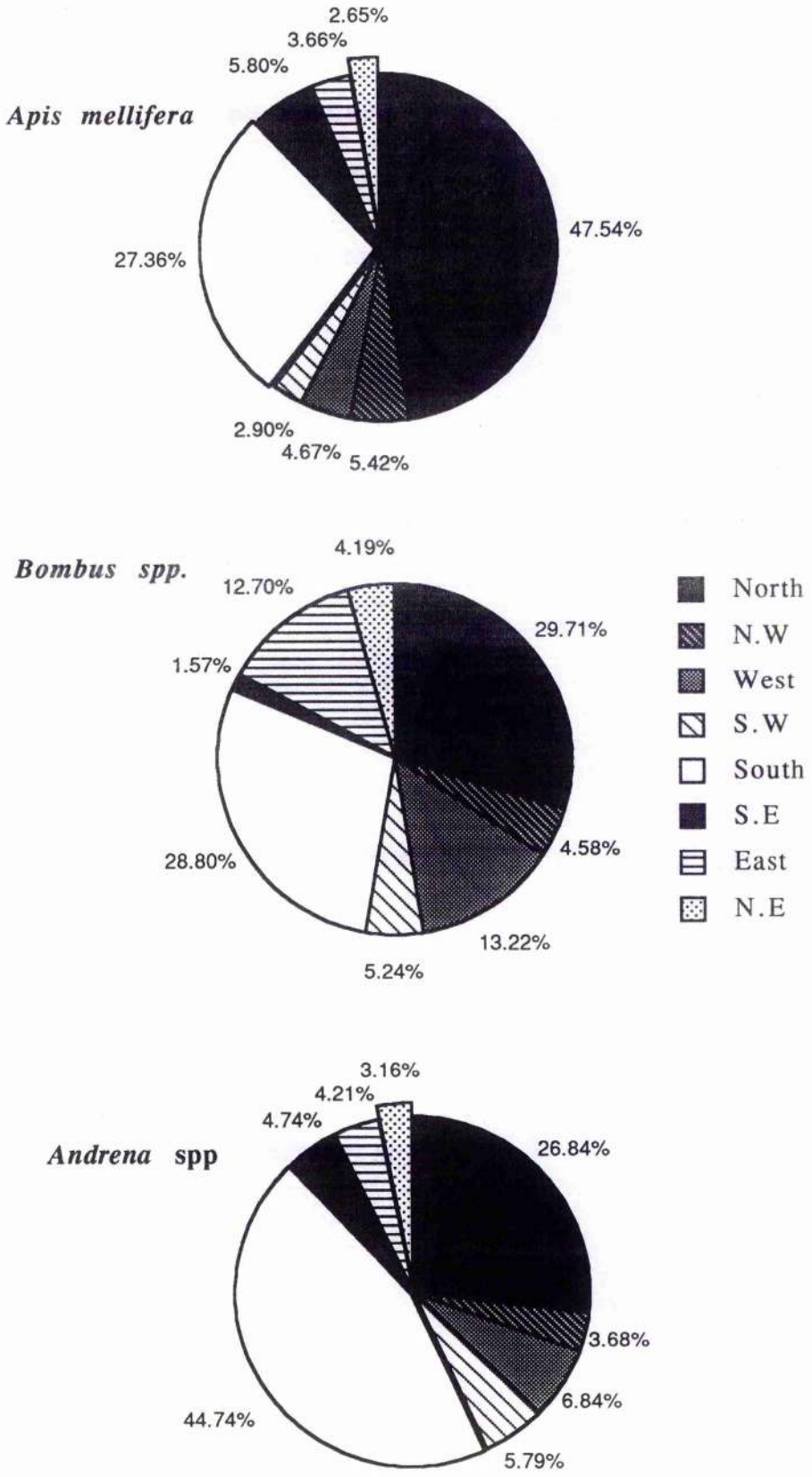

Fig. 7.2b. The percentages of bee's movements to different directions on Glen Prosen flowers. The raspberry rows run from north to south, while the E-W direction represents the movements to the adjacent rows.

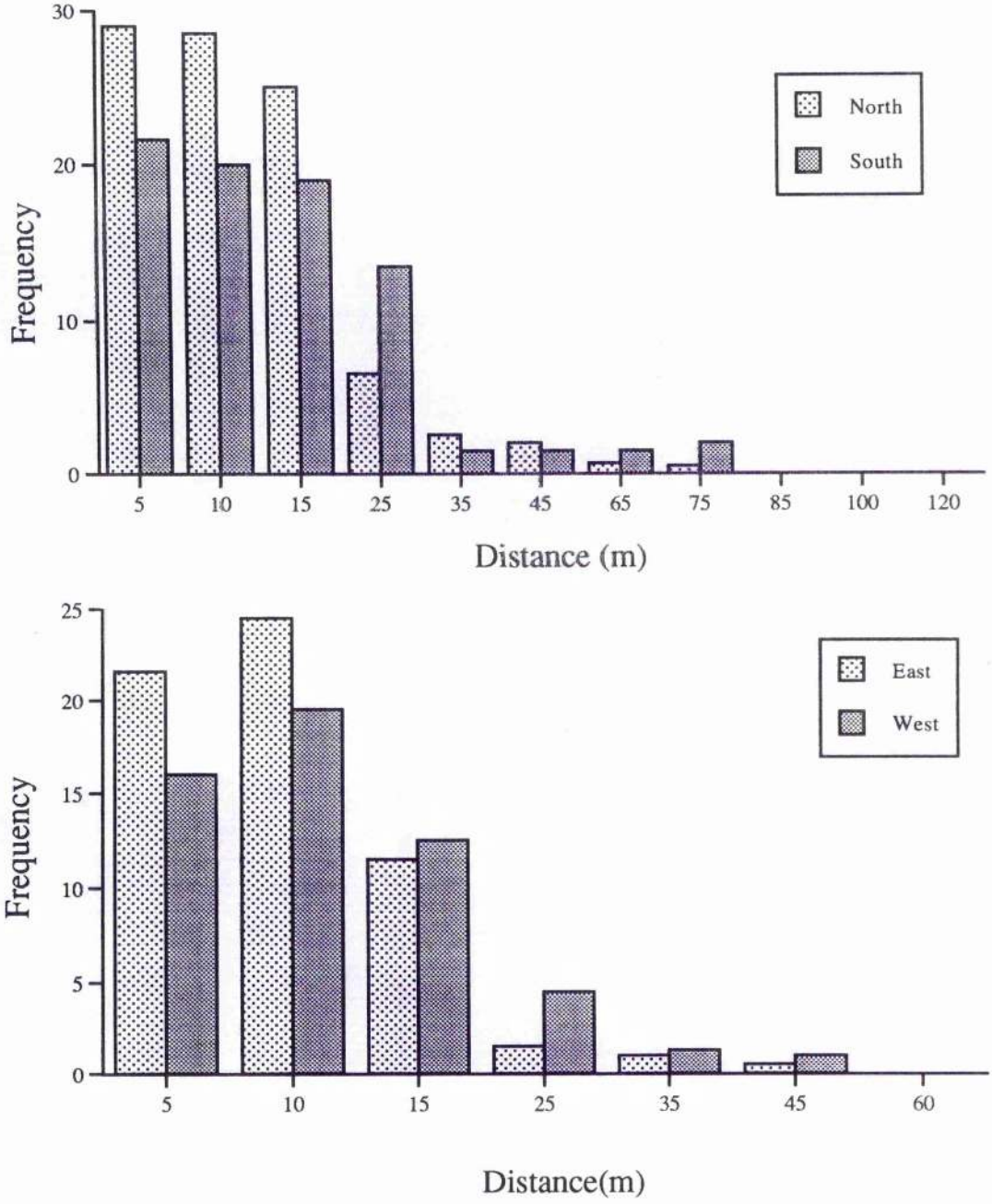

Fig. 7.3. The frequency of insect visitors movements to the different directions, transferring the pollen grains from the source (Glen Clova) to the recipient Glen Lyon. Note the different scales on each plot.

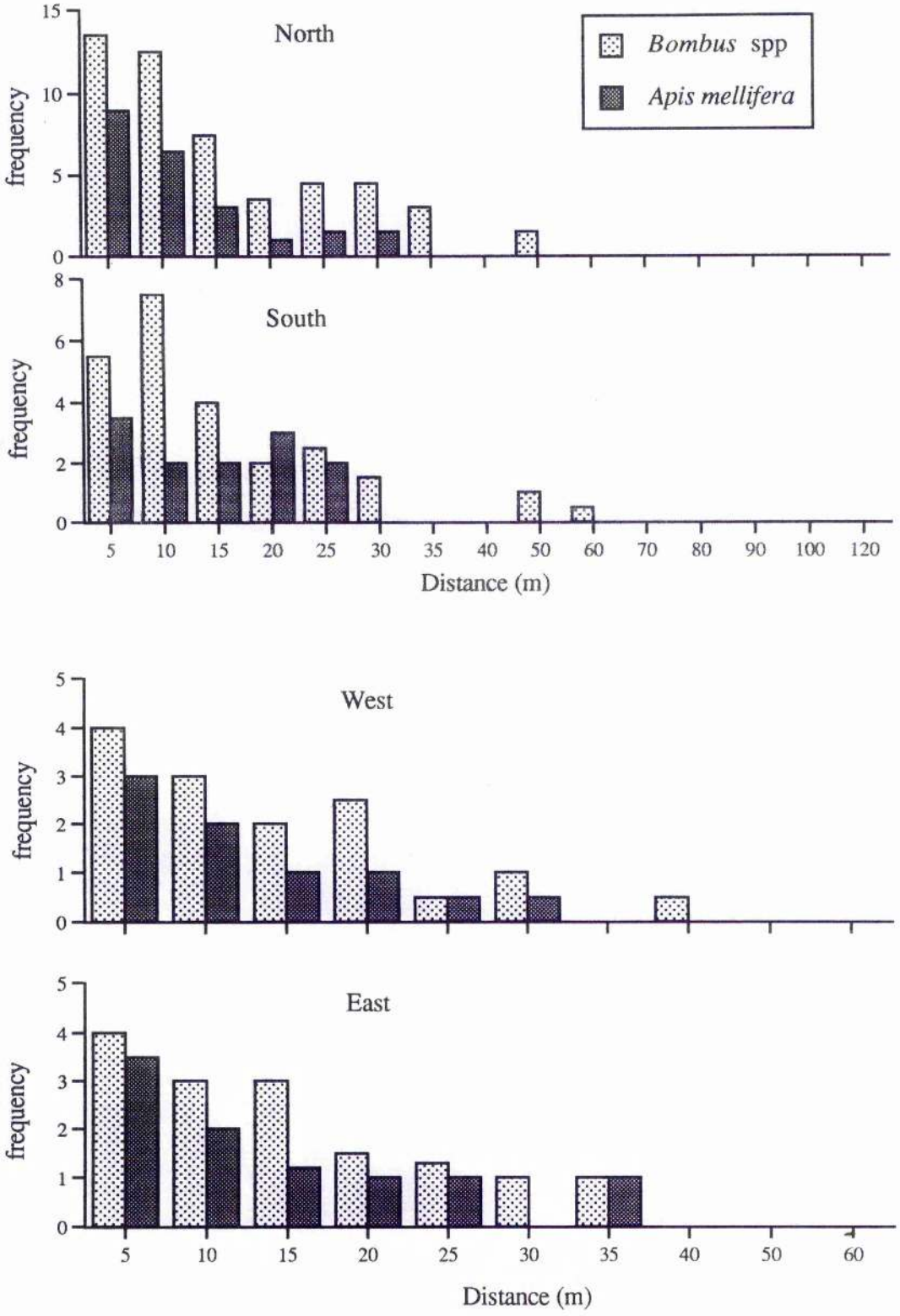

Fig. 7.4. The frequency of *Bombus* species and *Apis mellifera* movements to the different directions, transferring the pollen grains from the source (Glen Clova) to the recipient Glen Lyon. Note the different scales on each plot.

GENERAL DISCUSSION

This chapter brings together all the conclusions that have been reached on the pollination of raspberry plants and on their pollinators. The present study is the first comprehensive investigation comparing the foraging behaviour of cultivated and wild red raspberry's (*Rubus idaeus*) flower visitors, which documents the movement within and among these plants in Scotland. Only a few studies have previously attempted investigations of foraging behaviour of cultivated raspberry flower's visitors (Willmer, Bataw and Hughes 1994), while there appears to be no published information about the pollination of wild raspberry flowers.

Chapter 3 investigated the floral structure of the wild and cultivated raspberry flowers. Understanding floral structure and phenology is a prerequisite for understanding the floral life cycle as well as a necessary background for any pollination investigation (Dafni 1992). The raspberries studied showed very similar flower morphology; differences occurred only in flower diameter. Glen Moy flowers were largest, so that their nectaries could be more in number, surface area or volume than in the other cultivars, and this may be one of the reasons why Glen Moy produced higher amounts of nectar than Glen Prosen and wild raspberry (chapter 4). Glen Moy produced not only larger flowers but also more flowers per meter, and all these factors together might act to increase its attractiveness. All the cultivars provided most of their pollen grains on the first day, with decreased pollen as flower age increased. This means the young flowers could best provide the visitors with what they need, for their own food and or for their nest. In terms of pollination, the greatest chances of transfer of the pollen would also occur in the first stages of the flower's life.

The changes in nectar yield reflect a situation that is complicated by the interaction of a number of factors that influence the amount and concentration of nectar present in the flowers at any time. These include the nectaries' activity (secretion or reabsorption); the extent of post-secretory equilibration, likely to be high because the nectaries of raspberry are quite exposed so that the nectar they contain is probably greatly influenced by changes in the relative humidity of the atmosphere; and removal of nectar by insects.

The greatest effect of the microclimate on nectar solute concentration seems to be a direct effect of air humidity on the water content of nectar. This was assessed with different degrees of precision by Vansell (1940), Huber (1956), Corbet (1978a, 1978b), and by Corbet *et al* (1979a, 1979b), who gave a detailed account of the post-secretory changes of nectar concentration. In all the three raspberry cultivars studied, within-day variation in nectar concentrations correlated significantly with measured microclimatic variables. Between-day variation in mean values of nectar concentration also correlated significantly with relative humidity and temperature.

The importance of time of sampling and microclimate as determinants of nectar volume and nectar concentration has been ignored by many authors as an effect when considering the significance of nectar concentration for pollination (e.g. Baker *et al* 1975, Marden 1984). But my study agrees with Corbet *et al* (1979a) and Plowright (1981) who stressed that reports of field studies on nectar solute concentration are of enhanced value if they include information on relevant microclimatic changes such as temperature and humidity.

The next question that arises is, does removal or non removal of nectar from flowers affect the nectar production rate on the subsequent days of anthesis? The results (Table 4.3) show that there is a significant difference between repeat-sampled and once sampled flowers in the amount of nectar

produced at the end of a day 1800h; that is, repeat sampling could stimulate nectar tissues to further nectar production, even with maximum care taken to avoid damaging the flowers.

Only a few studies have investigated the effect of nectar removal on production rates (Raw 1953; Feinsinger 1978; McDade & Kinsman 1980; Plowright 1981). The results described here could explain why many insects, in particular *Bombus* species, strongly select young flowers (first day of anthesis) which offer high nectar reward, rather than old flowers which offer less nectar partly as a result of the effect of repeated visits on the first day of anthesis .

The differences between individual flowers in each raspberry cultivar in producing nectar were very large, and this parallels the situation in other plants (e.g. Pedersen 1953; Walker *et al* 1974; Lack 1982a). Such differences may be of selective importance (Lack 1982b). Rate of nectar intake by individual insect visitors can be affected by choices made at the individual flower level, because flowers in the individual plants are highly variable in the amount of nectar produced even beyond the variation among young and old flowers.

A result of particular interest was the wide variation in nectar concentration among the flowers even on the same branch of a raspberry plant during the course of this study, in both bagged and unbagged flowers. Other investigators have also reported considerable ranges in nectar concentration: 16 - 50% for orange blossom (Vansell 1952), 14 -68% for white clover, 10 - 37% for sweet clover, and 17 - 49% for goldenrod (Oertel 1944). Plowright (1979) suggested that the lower values in these ranges may be close to the concentration of freshly secreted nectar; though possible contamination by dew or rain must be always be considered. The nectar secretion variation from flower to flower within either cultivated or wild raspberry has been attributed to a number of factors: for example, the position of the flower on the plant, the age

of the flower and of the plant (Percival 1946; Proctor & Yeo 1973), and environmental factors (Shuel 1955a,b).

Chapter 5 investigated the daily and seasonal abundance of visitors on raspberry flowers, and the factors that affect this abundance. Seasonal patterns in visitation by insects also followed the abundance of raspberry blossom; peak insect visitors' abundance corresponding to peak flowering seasons in each year. The visitors appeared to be less selective about choices of individual raspberry flowers than they can be on other plant species, perhaps because the resource is so unusually abundant. The raspberry flowers attracted a diverse group of insect visitors, but the most important visitors were the *Bombus* species, *Apis mellifera*, *Andrena* species and hover flies. These visitors differed in their selection of raspberry flowers.

The dominant factors affecting bee visits in general appear to be nectar abundance, sugar concentration and chemical attractants (Martin & McGregor 1973). The positive effect of more nectar on the number of pollinator approaches and the number of visits per flower has been well documented (e.g. Pyke 1978a,b; Heinrich 1979; Waddington 1981). A flower that presents a greater nectar reward is more attractive for pollinators. At the same time, more nectar prolongs flower visitation sequences and more time is spent in each flower (Zimmerman 1983; Klinkhamer & de Jong 1993). Most of the insects which were attracted to Glen Moy, Glen Prosen and wild raspberry flowers fed primarily on nectar (chapter 6) and any difference between the cultivars in nectar production would therefore be detected by the insects and will determine which cultivars they visit most frequently. The insect visitors visited Glen Moy cultivar more frequently, as it produced more nectar and pollen grains than Glen Prosen and wild raspberry flowers. The results clearly also show a significant effect of number of flowers produced (patch size) in attractiveness to visitors in all cultivars; plants with more flowers were visited by more insect visitors. This

finding agrees with Willson & Bertin (1979) who suggest that the frequency of visitors increases with increase of inflorescence size of *Asclepias syriaca*.

Weather conditions influenced the diurnal rhythm of insect activity (Willmer 1982,1983). The numbers of *Apis mellifera* and *Andrena* species foraging were significantly affected both by temperature and relative humidity but were not greatly affected by nectar quality. *Bombus* species, by contrast, were affected by relative humidity, but not by temperature. Since *Bombus* species can warm up by metabolic means independent of solar radiation (Heinrich 1975b, Willmer 1983) they forage early in the day on all the three raspberry cultivars' flowers and to a lesser extent also forage in the evening when other visits have ceased. *Bombus* therefore respond primarily to reward quality (itself affected by relative humidity) and forage largely independently of ambient temperature.

The differences in response to temperature between *Apis mellifera*, *Andrena* species and *Bombus* species may be of importance for the reproductive success of raspberry plants which require insects for pollination, in places where cool summers may limit pollinator activity. Raspberry is traditionally best grown in areas with relatively cool summers, such as in Scotland, so that the importance of *Bombus* species as pollinators for raspberry plant may be a rather general phenomenon. Willmer, Bataw and Hughes (1994) suggested that these are important implications for the practicalities of raspberry growing and pollination, particularly in relation to encouraging bumble bees by retaining hedgerows as nest sites and sources of other foraging plants out of the raspberry season.

Observations of bee visitors to the three raspberry cultivars showed that the visits of each of them resulted in deposition of *Rubus* pollen grains on stigmas, although the insects varied in the number of grains that they carried.

Bombus species carried more pollen grains than *Apis mellifera* and *Andrena* species on all the three raspberry cultivars, and this can be explained partly by their larger body size. Most of the bees restricted their pollen gathering, as revealed by pollen carried on their bodies, more to the *Rubus* pollen than to the surrounding plant species.

Bombus species also showed preferences to forage on young flowers more than honey bees and *Andrena* species, and it is the young flowers from which pollen is available (chapter 3). They also visited rather more flowers per minute than *Andrena* or honey bees (Table 6.4).

Once alighted on a plant most of the bee movements were strongly directional. Bumble bees, *Apis mellifera* and *Andrena* species generally started to forage from the bottom point of raspberry canes and moved within the plant by flying from flower to flower moving up the cane, rather than down. Both bees and plants can get benefits from upward movement (Pyke 1978c). For the plant this is because the bee will increase cross-pollination, and for bees, because Pyke's study provided evidence that the amount of nectar in lower flowers was greater, so that a bee could maximize its energetic profit by starting low and working upwards. However, Corbet *et al* (1981) argued that the directionality of intra-inflorescence movements of insect visitors either upward or downward could depend in part on the position they adopt during their flower visits. My investigation agreed with Pyke's result; the amounts of nectar in raspberry flowers were greater in lower flowers than in the top ones. It has been demonstrated that the foraging movements of bees respond to changes in nectar availability (Hodges & Russell 1981b; Sowig 1989).

Optimal foraging theory predicts that movements of flower-visiting animals should be such that they maximize their net energy gain (see Pyke 1978a). Indeed, pollinators often forage within small areas, visiting neighboring

plants as a function of plant or flower density. Most of the common raspberry visitors tended to move in the same direction (north-south) as the rows of canes; this movement agreed with optimal foraging theory, as they would maximize their net energy when they move to the closest neighbour plants on the some row rather than move in another direction to the parallel rows. These movements should ensure that the amount of gene flow will be high within the same row, if there is pollen carry over.

Pollen flow was studied using fluorescent dyes. *Bombus* species and honey bees can transfer dye particles (pollen grain mimics) in different directions and different distances. Pollen was carried up to 60 meters by *Bombus* species and about 35 meters by honey bees. Pollen grains were carried by both insects in all directions though again primarily to North and South along rows; and most of the dye particles were deposited in the areas around the source plant (donor variety).

The exact evaluation of the importance of particular visitors on any crop as a result of their frequencies and abundance cannot be judged easily (Willmer, Bataw & Hughes 1994). Quantification of the relative effectiveness of pollinators is an inexact and complex issue (Heinrich & Raven 1972, Primack & Silander 1975, Motten *et al* 1981) but it must take into account a range of factors concerned with floral selectivity and constancy, seasonal patterns in relations to floral phenology, diurnal activity patterns in relation to floral dehiscence and stigmatic receptivity, flight directionality pattern and distances, and ultimately effectiveness of pollen carriage and transfer leading to correct pollen-tube growth (cf. Davis 1992).

However, from all the comparisons and results described, I can suggest that *Bombus* species are substantially better at transferring pollen between raspberry flowers and between patches of raspberry, than are honey bees and

Andrena species in wild and commercial areas of eastern Scotland. The following reasons can be highlighted:

1. The *Bombus* species are more abundant on both wild and cultivated raspberry, and make a high percentage (about 60%) of all the flower visits averaged over many days and several seasons.
2. They select younger flowers strongly more than do honey bees and *Andrena* species; and it is the young flowers from which pollen grains are available and between which they can be effectively transferred.
3. The *Bombus* species visit rather more flowers per minute than honey bees and *Andrena* species.
4. The *Bombus* species are more frequently present in the early mornings when pollen dehiscence is at a peak; *Andrena* species and honey bees were rare on most mornings of observation.
5. They carry significantly more pollen on their bodies.
6. They deposit significantly more pollen on stigmas in both cultivated and wild raspberry flowers.
7. They move the pollen grains over longer distances than honey bees.

However, it is worth stressing that bumble bees may not necessarily be the best pollinators even of commercial raspberry when it is grown in other sites. All pollinating insects are dependent upon microclimatic conditions, and bees despite their endothermic capabilities have specific minimum temperatures for activity (Stone & Willmer 1989) leading to specific 'microclimatic windows' in which they can behave effectively as pollinators (Corbet *et al* 1993). Honey bees are generally more active, and may be active earlier in the day, in the warmer weather prevailing in southern Britain (Willmer 1983). The raspberries grown in southern areas may themselves show different dehiscence pattern, and different nectar properties, or even different flowering phenology, either because of warmer sites or because different cultivars are preferred; these factors could substantially alter the relations between flowers and bee visitors.

However commercial raspberry is traditionally best grown in areas with relatively cool summer, with largest acreages in Scotland, north-east Europe, and Canada, so that the importance of non-*Apis* bees as pollinators may indeed be a rather general phenomenon.

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